Hepatocarcinogenicity of Dehydroepiandrosterone in the Rat

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Abstract

Dehydroepiandrosterone, a major secretory steroid hormone of the human adrenal gland, possesses mitoinhibitory and anticarcinogenic properties. It also induces peroxisome proliferation in the livers of rats and mice. Because peroxisome proliferators exhibit hepatocarcinogenic potential, it is necessary to examine the long term hepatic effects of dehydroepiandrosterone since this hormone is contemplated for use as a potential cancer chemopreventive agent in humans. Dehydroepiandrosterone was administered in the diet at a concentration of 0.45% to F-344 rats for up to 84 weeks. At the termination of the experiment, 14 of 16 rats developed hepatocellular carcinomas. Liver tumors induced by dehydroepiandrosterone lacked γ-glutamyl transpeptidase and glutathione S-transferase (placenta! form); these phenotypic properties are identical to the features exhibited by liver tumors induced by other peroxisome proliferators. Dehydroepiandrosterone was also shown to markedly inhibit liver cell [3H]thymidine labeling indices, suggesting that cell proliferation is not a critical feature in liver tumor development with this agent. These results show that although dehydroepiandrosterone exerts anticarcinogenic effects in a variety of tissues, the peroxisome-proliferative property makes it a hepatocarcinogen.

Introduction

DHEA, a naturally occurring adrenal steroid hormone, has been shown to exert a protective role against initiation and promotion stages of carcinogenesis in experimental animals (1, 2). The anticarcinogenic effect of DHEA is attributed primarily to the inhibition of glucose-6-phosphate dehydrogenase activity resulting in the reduction of cellular NADPH pool (1, 3). Decreased NADPH pool leads to low levels of mixed function oxidases, enzymes that participate in the activation of procarcinogens to ultimate carcinogens, and nucleotides that are needed for cell proliferation. Recent studies have shown that DHEA elicits pleiotropic responses in the rat liver characterized by hepatomegaly, peroxisome proliferation, induction of peroxisome-associated enzymes, and microsomal enzymes (4–7). These hepatic changes caused by DHEA are very similar to those induced by certain hepatocarcinogenic xenobiotics referred to as peroxisome proliferators (8). Since it is well established that peroxisome proliferators are hepatocarcinogens in rats and mice (8, 9), the question arises about the possible paradox of the well documented anti-proliferative and antineoplastic properties of DHEA and the implication that it may in fact induce liver tumors by virtue of its peroxisome-proliferative property. Although the mechanism by which peroxisome proliferators, which are proved nongenotoxic and nonmutagenic chemicals, induce liver tumors remains controversial, we have postulated that oxidative stress and the ensuing oxidative DNA damage as the most rational mechanism responsible for peroxisome proliferator-induced hepatocarcinogenesis (10). Others have postulated that cell proliferation may be responsible for peroxisome proliferator-induced carcinogenicity (11) or that these agents act by exerting a promotional effect on the so-called “spontaneously initiated” liver cells (12). Since DHEA has been shown to be a potent anticarcinogen in certain experimental animal tumor model systems, including its ability to inhibit spontaneous cancer formation (1, 13) and also that it was demonstrated to possess a profound antimitotic potential (1, 3), it provides a valid tool with which to examine the relationship of peroxisome proliferation and liver cancer. The results unequivocally demonstrate that the anticarcinogenic and antimitotic DHEA is a hepatocarcinogen in the rat, most likely by virtue of its peroxisome proliferative pleiotropic properties.

Materials and Methods

Fifteen-week-old male F-344 rats, weighing 250–275 g, were purchased from Charles River Breeding Laboratories (Wilmington, MA). Rats were housed in rooms of 3 to 4 in polycarbonate cages containing San-i-Cel bedding in a temperature- and humidity-controlled room with a 12-h dark-light cycle. Sixteen rats were fed AIN-76 semipurified diet (corn oil stripped of vitamin E; US Biochemical Corp., Cleveland, OH) containing 0.45% w/w dehydroepiandrosterone acetate (Sigma Chemical Co., St. Louis, MO). Ten rats were maintained on a control diet without DHEA. All rats had free access to food and water. Three DHEA-treated rats were killed between 70 and 75 weeks, and the remaining 13 rats were killed at 84 weeks. Two control rats were killed at 70 weeks and the remaining controls were killed at 84 weeks. A complete necropsy was performed on all rats. Livers were excised and examined for gross lesions after serial sectioning. Selected portions of liver were fixed in 10% neutral buffered formalin and processed for routine histological examination. In addition, some sections of the liver were fixed in ethanol-acetic acid and processed for histochemical localization of GGT (14). Sections from lungs, pancreas, and kidneys were also processed for light microscopy. Paraffin sections (4–5 μm thick) were stained with hematoxylin and eosin, and those containing neoplastic lesions were stained for GST-P by immunoperoxidase method (14). To study the effect of DHEA on liver cell proliferation, we have injected a single dose of [3H]thymidine (1 μCi/g body weight), 1 h prior to sacrifice to control rats and rats fed DHEA for 4, 7, or 14 days. Another group of rats fed DHEA for 24 weeks and age-matched controls were also used for [3H]thymidine labeling. Sections of liver were autoradiographed and the labeled nuclei were counted. Portions of liver were also processed for DNA extraction and scintillation counting.

Results and Discussion

Body and liver weights and incidence of liver tumors are summarized in Table 1. As expected, DHEA treatment caused a marked reduction in body weights when compared to controls. The liver weight: body weight ratios were significantly different versus 6.5 g/100 g body weight, although the differences in

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: DHEA, dehydroepiandrosterone; GGT, γ-glutamyl transpeptidase; GST-P, glutathione S-transferase placental form (GST 7–7); HCC, hepatocellular carcinoma; NN, neoplastic nodules.

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absolute liver weights were not as marked between the two groups. The observed increases in absolute liver weight in animals fed DHEA are attributable, in part, to the presence of tumors. Grossly, livers of 15 of 16 rats treated with DHEA for 70 to 84 weeks showed one or several gray-white lesions, some as large as 25 mm in diameter (Fig. 1). The liver of one DHEA-fed rat that did not contain any gross tumors was infiltrated with leukemic cells. Livers of all the other rats contained altered areas, NN and HCC. Cells in altered areas were larger than adjacent parenchymal cells and exhibited eosinophilic or basophilic cytoplasm with an increased nuclear cytoplasmic ratio and prominent nucleoli. Cells in NN usually displayed morphological features similar to those of cells in altered areas. However, in NN the mitotic activity was very prominent, with cells arranged in 2–3-cell-thick plates and causing compression of adjacent parenchyma. HCC were well differentiated with trabecular, solid sheets or pseudoglandular patterns (Fig. 2). Tumor cells displayed cytological and nuclear features of low grade hepatocellular carcinomas. Mitotic activity was similar to that seen in NN. Extension of tumors into vascular spaces or metastasis to lungs was not observed. All types of liver lesions, i.e., altered areas, NN and HCC, that were examined for GGT and GST-P lacked these 2 marker enzymes (Fig. 3). Livers from control animals did not show any grossly visible tumors. Histologically, an occasional altered area was present. In 2 control rats there was involvement of liver with acute leukemic infiltration.

The results of this study unequivocally demonstrate that DHEA, a compound generally considered anticarcinogenic with inhibitory effects on cell replication, is a hepatocarcinogen in rats. Ninety-four % of rats developed neoplastic lesions including HCC in 88%. No liver tumors were observed in control rats that were fed the same diet without DHEA. The morphological and phenotypic properties of DHEA-induced liver lesions are essentially similar to those induced by classical chemical peroxisome proliferators (7, 14). Lack of GGT and GST-P is unique to peroxisome proliferator-induced liver tumors in contrast to genotoxic carcinogen-induced liver tumors in which these markers are consistently expressed (15). This difference in the phenotypic properties of peroxisome proliferator-induced and genotoxic carcinogen-induced lesions has been attributed to possible differences in the mechanism of action of these 2 classes of carcinogens (16).

The development of liver tumors in rats treated with this widely regarded anticarcinogenic, DHEA, although it appears surprising, is not totally unexpected because of the ability of DHEA to increase the number of peroxisomes in liver parenchymal cells (4–6). Recent studies from our laboratory have shown that DHEA at a dietary concentration of 0.45% caused a 5-fold increase in the volume density of peroxisomes and a 2- and 12-fold increase in the levels of catalase and peroxisomal bifunctional enzyme mRNAs, respectively (5), in agreement with the suggested mechanism by which peroxisome proliferators lead to oxidative stress. Catalase, the enzyme which degrades H₂O₂, is minimally induced, whereas induction of per-
oxisomal β-oxidation system enzymes leads to the generation of H₂O₂ several orders of magnitude higher than that in normal liver (17). Data from several earlier studies have clearly established that peroxisome proliferators are not capable of causing DNA damage directly but they most likely induce DNA damage indirectly through receptor-mediated biological actions (16, 18). In general, the potency of tumor induction by peroxisome proliferators has been correlated well with their capacity to induce sustained proliferation of peroxisomes (19). From previous published studies and this present study it is apparent that DHEA at the dose level used in this study ranks intermediate in potency in inducing peroxisomes when compared to ciprofibrate and di(2-ethylhexyl)phthalate.

The results of incorporation of labeled thymidine into liver DNA and labeling index of hepatocyte nuclei in DHEA-treated and control rats are presented in Table 2. A decrease in thymidine uptake and the number of labeled nuclei is evident in DHEA-treated rats.

Liver cell proliferation has been suggested to play a primary role in tumorigenicity of peroxisome proliferators (11). The results of the present study show that liver cell proliferation in vivo is inhibited by DHEA which tends to negate the hypothesis that cell proliferation is the basis for peroxisome proliferator carcinogenesis. DHEA was shown to inhibit DNA synthesis in both in vitro and in vivo experiments (1, 3). Finally, the non-genotoxic nature of peroxisome proliferators prompted the suggestion that peroxisome proliferators act as promoters of spontaneously initiated liver lesions by virtue of the mitogenic potential of these chemicals (12). The fact that DHEA does not induce cell proliferation in liver suggests that promotion of the so-called spontaneously initiated cells may not be the mechanism in DHEA-induced hepatocarcinogenesis. It is worth noting that DHEA has been shown indeed to inhibit the spontaneous breast cancer formation in mice (13). These data strongly support our view that enormous increases in the free radical-generating peroxisomal enzymes can cause initiation albeit slowly and that such cells can then escape from or become resistant to the peroxisome proliferators (20). If normal endogenous levels of oxidative stress are accepted as the reason for the presumed spontaneous initiation (12, 21), then why would not the well documented remarkable increases in the levels of hydrogen peroxide-generating peroxisomal fatty acyl-CoA oxidase in livers be responsible for increased rates of initiation and tumorigenesis in animals with peroxisome proliferator-induced chronic and sustained oxidative stress? DHEA presumably elicits its biological effects by interacting with a receptor, similar to the mechanism proposed for the transcriptional activation of β-oxidation system genes by peroxisome proliferators (17, 18). Thus, these nongenotoxic agents provide important tools for elucidating the mechanisms of receptor mediated hepatocarcinogenesis (16, 18).

**DHEA HEPATOCARCINOGENESIS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Liver wt/100 g body wt [³H]Thymidine incorporation (dpm/µg DNA)</th>
<th>Labeled nuclei/1000 hepatocyte nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DHEA for 4 days</td>
<td>7.4 ± 0.1 ± 0.3</td>
<td>135 ± 11</td>
</tr>
<tr>
<td>2</td>
<td>Controls</td>
<td>5.3 ± 0.1</td>
<td>127 ± 15</td>
</tr>
<tr>
<td>3</td>
<td>DHEA for 7 days</td>
<td>8.0 ± 0.2</td>
<td>64 ± 12</td>
</tr>
<tr>
<td>4</td>
<td>DHEA for 14 days</td>
<td>8.3 ± 0.2</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>Controls</td>
<td>4.4 ± 0.3</td>
<td>4.65 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>DHEA for 24 wk</td>
<td>4.5 ± 0.2</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>7</td>
<td>Controls</td>
<td>3.0 ± 0.1</td>
<td>37 ± 2</td>
</tr>
</tbody>
</table>

*Each group contained 4 to 5 rats. DHEA was mixed in diet at a concentration of 0.45%.

References


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