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Phenotypic Properties of Liver Tumors Induced by Dehydroepiandrosterone in F-344 Rats

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Dehydroepiandrosterone (DHEA), a C19 adrenal steroid hormone, induces peroxisome proliferation in liver cells and is hepatocarcinogenic in the rat. The present study deals with the phenotypic properties of DHEA-induced liver lesions. A majority of the altered areas (80–87%), neoplastic nodules (>94%) and hepatocellular carcinomas (HCC, 80–100%) lacked the marker enzymes γ-glutamyltranspeptidase and placental form of glutathione *S*-transferase (GSTP). Northern blot analysis of HCC from 4 rats revealed no detectable GSTP mRNA. These HCC, however, showed a marked decrease in the staining of glucose-6-phosphatase and adenosine triphosphatase. These results indicate that the phenotypic properties of liver tumors induced by DHEA and amphipathic carboxylate peroxisome proliferators are similar.

Key words: Hepatocellular carcinoma — Oxidative stress — Peroxisome — Glutathione *S*-transferase P

The preneoplastic and neoplastic liver lesions induced by genotoxic hepatocarcinogens display phenotypic features different from those of normal liver cells. These properties include loss of some normally present enzymes (G6Pase,[2] ATPase, drug-metabolizing enzymes, etc.) and acquisition of some new enzymes and proteins (GGT, GSTP, AFP, etc.).[1–6] GGT and GSTP are consistently expressed in genotoxic carcinogen-induced lesions and are considered as the most reliable marker enzymes for identification of preneoplastic and neoplastic liver lesions.[3,7] In contrast, the liver lesions induced by a class of non-genotoxic hepatocarcinogens designated as PPs usually lack these marker enzymes and AFP.[8–10] The absence of these enzymes and AFP in PP-induced tumors is due to lack of their mRNAs.[11] The peculiar phenotypic properties of PP-induced lesions may be closely related to the mechanism of action of these non-genotoxic carcinogens or activation of a peroxisome proliferator-associated receptor which may interfere with transcriptional activation of some oncogenes that regulate the expression of marker enzymes.[12–15]

DHEA, a naturally occurring adrenal cortical steroid, is a novel member of the so-called peroxisome proliferators. The hepatic pleiotropic effects induced by DHEA in rats and mice are similar to those induced by other well-studied PPs.[16] These effects include hepatomegaly, peroxisome proliferation, and induction of peroxisome-associated enzymes and microsomal enzymes.[16–20] DHEA differs from all the other PPs in that it lacks a carboxylic function. It appears that a metabolite of DHEA, not the parent compound, is responsible for the peroxisome-proliferative effect.[13,21,22] We have established that this hormone is a hepatocarcinogen.[23]

The purpose of this study was to analyze quantitatively DHEA-induced liver tumors for phenotypic alterations and to compare the phenotypic properties with those of tumors induced by other peroxisome proliferators and genotoxic carcinogens. Histochemical, immunochemical and mRNA studies have shown that DHEA and other PP-induced liver tumors exhibit identical phenotypic characteristics.

MATERIALS AND METHODS

Induction and enzyme analysis of tumors Preneoplastic and neoplastic liver lesions were induced in 15 male F-344 rats as described before.[23] Briefly, rats were fed AIN-76 semipurified diet containing 0.45% w/w dehydroepiandrosterone acetate (Sigma Chemical Co., St. Louis, MO) for 70–84 weeks. At necropsy some portions of liver containing grossly visible lesions were fixed in 10% neutral buffered formalin for routine histological examination and others were fixed in cold 95% ethanol-acetic acid and processed for histochemical localization of GGT.[24,25] For immunochemical localization of GSTP, 5 μm sections from formalin and ethanol-acetic acid fixed tissues were stained by avidin-biotin-

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[2] The abbreviations used are: AA, altered areas; AFP, α-fetoprotein; ATPase, adenosinetriphosphatase; DHEA, dehydroepiandrosterone; G6Pase, glucose-6-phosphatase; GGT, γ-glutamyltranspeptidase; GSTP, glutathione *S*-transferase, placental form; HCC, hepatocellular carcinoma; NN, neoplastic nodules; PPs, peroxisome proliferators.

peroxidase complex using GSTP antibodies raised in rabbits.[11] In addition, from 5 animals, portions of tumors (histologically proven neoplastic nodules or HCC) were snap-frozen in liquid nitrogen and stored at −70°C for histochemical evaluation of ATPase and G6Pase. Ten-micrometer frozen sections were cut and stained for G6Pase and ATPase by the method of Wachstein and Meisel.[26]

RNA extraction and Northern blot analysis Total RNAs were isolated from HCC (from 4 animals) by the method of Chomczynski and Sacchi.[27] The RNA was analyzed by Northern blot analysis using nick-translated ^{32}P-labeled GSTP cDNA.[11]

RESULTS

The livers of all rats contained lesions that were classified as AA, NN and HCC. A total of 10 AA, 16 NN, 16 HCC and 10 AA, 50 NN and 39 HCC were analyzed for GGT and GSTP, respectively. The results of the quantitative analysis are presented in Table I. Ninety to 100% of AA, NN and HCC were negative for GSTP. Similarly a large percentage of these lesions (80 to 94%) were also completely negative for GGT (Figs. 1 and 2). A small number of lesions in all categories showed scattered, weakly positive areas.

Histochemical stains NN and HCC (a total of 6) showed a marked decrease in the staining reaction for G6Pase in comparison to the adjacent liver (Fig. 3). Similarly these lesions also showed a decreased reaction for ATPase (Fig. 4).

GSTP mRNA levels The results of Northern blot analysis of total RNA obtained from four HCC are shown in Fig. 5. For comparison, RNAs isolated from 2-acetylaminofluorene-induced tumors were run in palallel. All DHEA-induced tumors were negative for GSTP mRNA, whereas AAF-induced tumors were positive.

Table I. Quantitative Analysis of Preneoplastic and Neoplastic Lesions Induced by Dehydroepiandrosterone for GGT and GSTP

	GGT		GSTP	
	±[a]	−	±	−
Altered areas	2 (20)[b]	8 (80)	1 (10)	9 (90)
Neoplastic nodules	1 (6)	15 (94)	1 (2)	49 (98)
Hepatocellular carcinoma	3 (19)	13 (81)	0	39 (100)

a) ± focally positive.
b) Numbers in parentheses are percentages.

DISCUSSION

PPs are a structurally diverse group of chemicals with the common property of peroxisome induction in the liver.[28] The chemical structure requirements for peroxisome induction are not clear. However, it is suggested that carboxyl groups or hydrophobic anion may be essential for peroxisome induction.[29, 30] Interestingly, the newly recognized peroxisome proliferator DHEA is structurally completely different from previously recognized PPs and has no known functional carboxylic or anionic groups. All PPs, including DHEA, so far tested for carcinogenicity in long-term experiments, have been shown to be carcinogenic in rats and mice.[20, 28] Since PPs are nonmutagenic, the carcinogenicity of these compounds was attributed to their ability to induce peroxisomes and peroxisomal enzymes by a receptor-mediated action, eventually leading to oxidative stress.[31–33]

Results of histochemical, immunochemical and Northern blot analysis in this study revealed that DHEA-induced liver tumors exhibited phenotypic properties identical, both qualitatively and quantitatively, to those induced by ciprofibrate-, WY-14, 643- and di(2-ethylhexyl)phthalate-induced tumors.[8, 11, 24] One hundred percent of HCC and 98% NN were negative for GSTP. Similarly 81% of HCC and 94% nodules lacked GGT. Even in the positive lesions the staining was seen only focally. Eighty to 90% of AA were also devoid of both GGT and GSTP.

The absence of GSTP proteins in DHEA-induced liver tumors is consistent with the absence of the mRNA. None of the four tumors (from 4 animals) evaluated by Northern blot analysis expressed GSTP mRNAs. Similar findings were observed with tumors induced by other PPs.[11] These findings are in contrast to what has been observed in tumors induced by genotoxic carcinogens.[3, 7, 11, 34, 35] The reason(s) for such striking differences in the expression of GGT and GSTP in tumors induced by PPs and genotoxic carcinogens is not fully understood. Such diverse phenotypic properties may reflect differences in the mechanism of action by these two groups of carcinogens.

Absence of GGT expression in PP-induced liver tumors may be due to lack of cytotoxic effects of these compounds, thus necessitating no adaptive changes. It was postulated PP-initiated cells are not depleted of glutathione and hence do not require GGT induction.[3] Suppression of GSTP expression may be related to interaction of peroxisome proliferator-activated receptor and c-*jun*. Recent studies have shown repression of glucocorticoid receptor activity by direct interaction between glucocorticoid receptor and JUN.[14] *In vitro* studies have shown a correlation between the levels of GSTP and c-*jun* mRNAs, indicating a possible regulatory

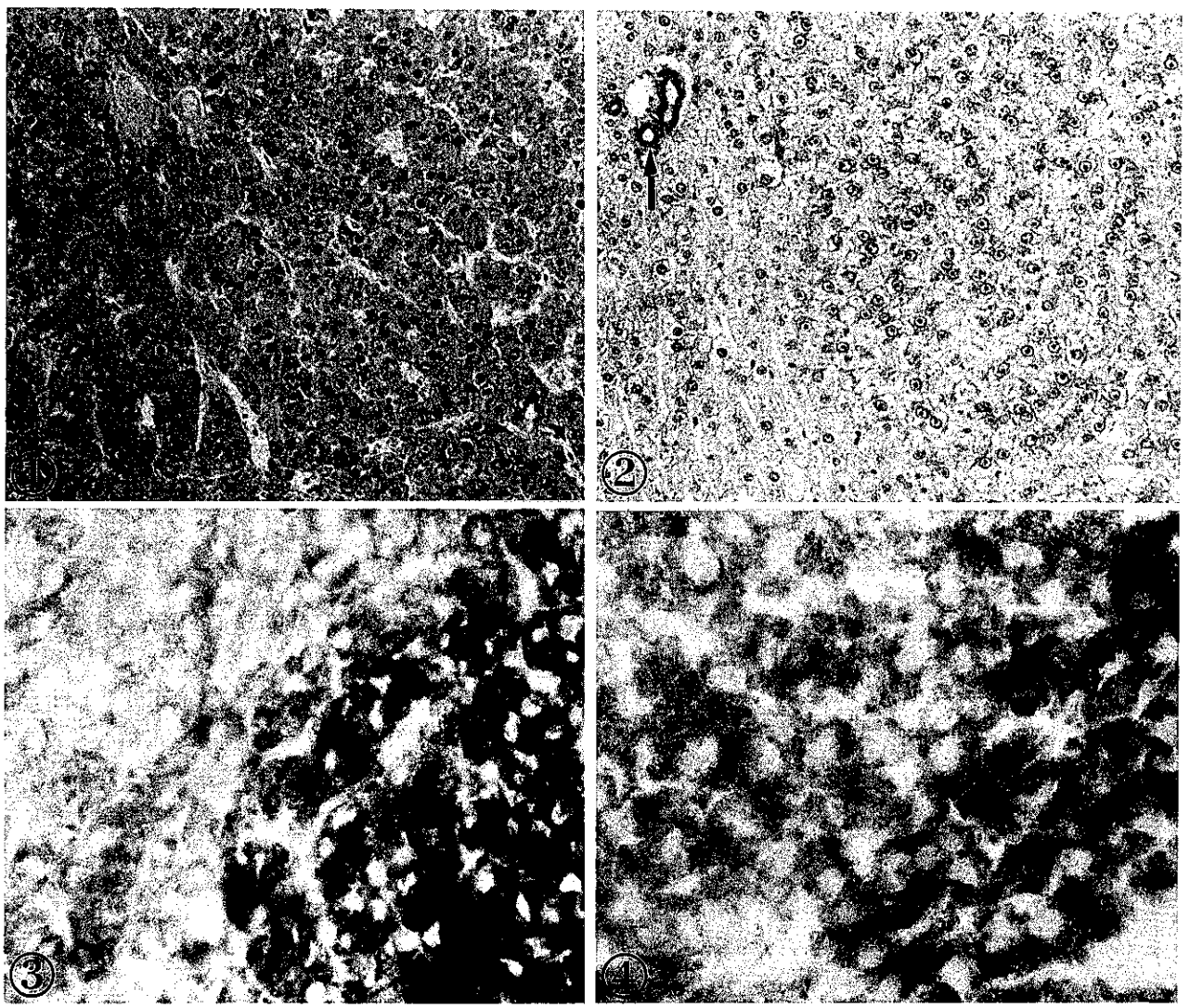

Fig. 1. Hepatocellular carcinoma stained for GSTP by the immunoperoxidase method. The tumor is negative for this protein. ×220.

Fig. 2. Neoplastic nodule stained for GGT by histochemical procedure. No GGT activity is present in the nodule. Adjacent bile ducts (arrow) are strongly positive. ×220.

Figs. 3 and 4. Hepatocellular carcinoma stained for G6Pase (3) and ATPase (4) by histochemical procedures. The tumor lacks these enzymes, whereas the adjacent liver shows intense staining. ×240.

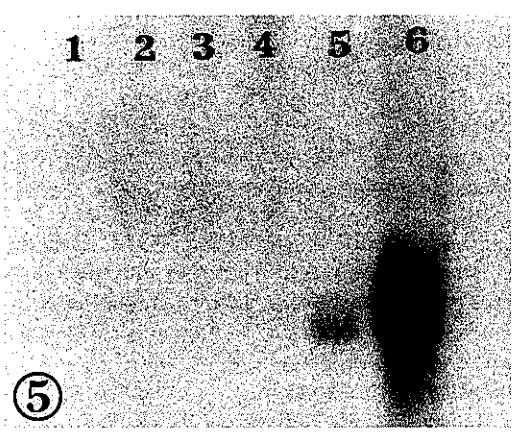

Fig. 5. Northern blot analysis of total RNA from HCC induced by DHEA (lanes 1–4) and AAF (lanes 5 and 6) probed with ^{32}P-labeled GSTP cDNA. The autoradiogram demonstrates 0.75 kb mRNA only in AAF tumors, but not in DHEA tumors. Each lane contains 10 μg of RNA.

effect of JUN on GSTP expression.[15] Studies by Sakai *et al.*,[36] have shown increased levels of c-Jun and GSTP mRNAs in HCC induced by genotoxic carcinogens. It will be of interest to examine PP-induced liver tumors for expression of c-*jun* mRNA and to see if there is any correlation with the absence of GSTP. It is of interest to point out that a high percentage of spontaneously developed liver lesions in female F-344 rats was negative for both GGT and GSTP.[37] The role of female sex hormones in negative enzyme properties of spontaneously developed liver lesions needs further investigation.

In summary, tumors induced by DHEA, a newly recognized structurally novel peroxisome prolferator exhibit identical phenotypic features with the tumors induced by other peroxisome proriferators. The peculiar phenotypic properties of these PP-induced tumors may reflect different mechanisms involved in receptor-mediated hepatocarcinogenesis.

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