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Hepatic Estrogen and Progesterone Receptors in an Estrogen-Associated Hepatic Neoplasm*

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Summary. *A patient with a benign hepatic neoplasm developing after treatment with estrogenic hormones is described. After excision, the neoplastic tissue was analyzed for the presence of cytosol estrogen and progesterone binding proteins. The neoplasm was classified as focal nodular hyperplasia of the liver and was demonstrated to contain high-affinity cytosol estrogen and progesterone hormone receptors. The estrogen-binding affinity of the neoplasm was three times greater than that of normal liver.*

Further investigation of cytosol hormone receptors in estrogen associated hepatic neoplasms will be required to define the role of these binding proteins in the possible etiology of certain liver tumors.

Introduction

Recently much attention has been focused on the possible association of exogenous estrogen administration and the induction of hepatic tumors in young women [2, 3, 5, 11, 14]. Both benign nodular hyperplasia and adenomas (2, 3, 11, 14) and malignant hepatocellular tumors [5, 11, 14] have been reported in patients who had taken oral contraceptives. Although statistical proof of the relationship between oral contraceptives and an increased incidence of hepatocellular neoplasia is not available, a number of series [3, 11, 16] have presented presumptive evidence supporting this hypothesis.

The possible mechanisms by which exogenous estrogens may induce or control the growth of hepatocellular neoplasia are unknown. However, it is probable that the presence of a high concentration of cytoplasmic receptor for estrogenic hormone would be a basic requirement. It is known that these receptors are important in predicting response to hormonal therapy in human breast cancer where their presence correlates with the probability of a positive reponse of the tumor to hormonal treatment [12]. We reasoned that cytosol estrogen receptors might be increased in estrogen-related hepatic tumors where their presence would be consistent with the possibility of estrogen stimulation of the neoplasm. In this communication we report the results of estrogen and progesterone receptor analysis on an estrogen-associated hepatic neoplasm.

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Materials and Methods

Case Report. A 53-year-old premenopausal Caucasian female underwent hysterectomy and oophorectomy for menorrhagia secondary to endometriosis at another hospital. At the time of abdominal exploration, a polypoid vascular tumor 8 × 8 cm in size and originating from the right lobe of the liver was noted. The patient was subsequently referred for evaluation and treatment of the hepatic tumor. 23 years prior to admission the patient had been placed on weekly intramuscular injections of diethylstilbestrol for 1 year to control menstrual irregularity. 10 years prior to admission, she noted a palpable mass in the right flank. An intravenous pyelogram was reported as normal. 5 years prior to admission, the patient had taken norethynodrel with mestranol (Enovid) as an oral contraceptive for 3 months. At the time of her evaluation at this hospital, a firm, 6-cm mass could be palpated below the right costal margin lateral to the mid-clavicular line, which moved well with respiration. The remainder of her physical examination was essentially unremarkable. Laboratory evaluation revealed normal complete blood count and urinalysis. The serum alkaline phosphatase was 1.6 Bessey-Lowry units/100 ml (normal < 2.5), SGOT was 35 IU, and SGPT was 31 IU/100 ml (normal < 35 IU). Bilirubin was 0.2 mg/100 ml direct and 0.5 mg/100 ml total. Prothrombin time and partial thromboplastin time were both within normal limits. The plasma CEA was 2.9 ng/ml (normal < 2.5 ng/ml) and plasma alpha-feto-protein was normal (< 40 ng/ml). The hepatic scan showed a round mass, which concentrated technetium and projected from the inferior surface of the right lobe of the liver. Selective arteriography demonstrated a vascular mass with its main blood supply derived from the right hepatic artery. The relatively increased vascularity of the lesion may have ac-

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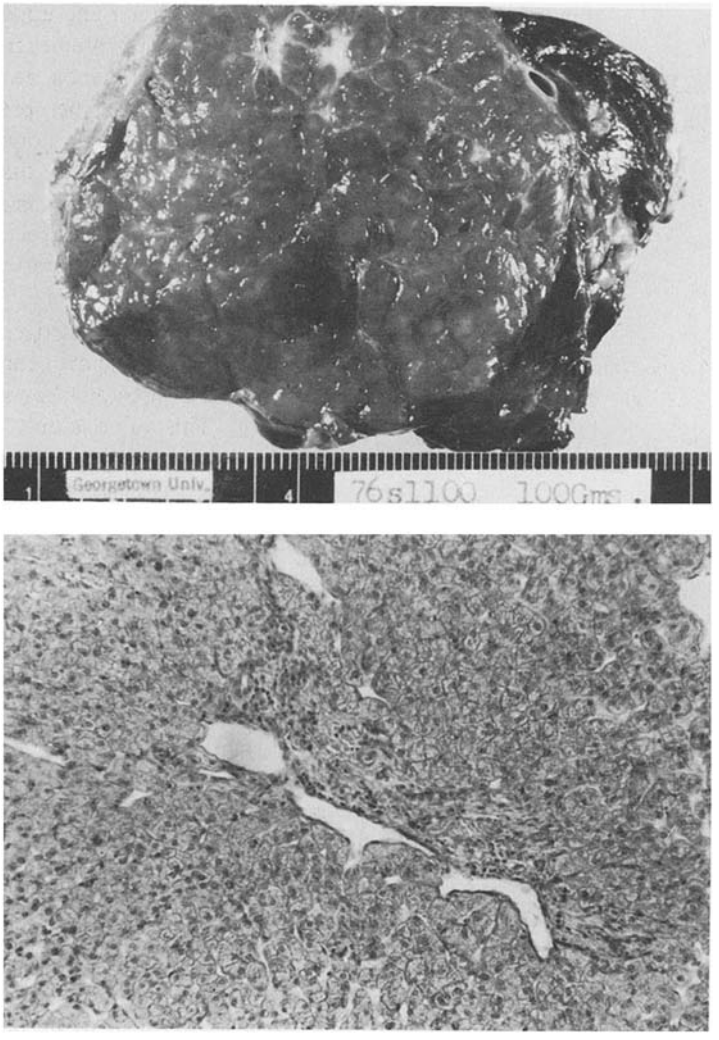

Fig. 1. Gross and microscopic appearance of tumor is consistent with diagnosis of focal nodular hyperplasia of liver. **A** Macroscopic observation reveals that tumor is composed of nodules of hepatic tissue separated by numerous fibrous trabeculae; **B** Microscopic examination of tumor reveals nodules of hepatocytes with no portal tracts within the nodules. However, there is proliferation of bile ductules within fibrous septae of tumor. Vascular elements also present in fibrous trabeculae

counted for the significant uptake of technetium. Intravenous pyelogram and upper and lower gastrointestinal series were all within normal limits. A wedge resection of the lesion was subsequently performed without complication. The gross and microscopic findings were consistent with focal nodular hyperplasia of the liver (Fig. 1). A sample of the neoplasm was assayed for the presence of estrogen and progesterone receptors.

Results

Estrogen receptor studies on the 100,000 *g* supernatant cytosol prepared from the tumor homogenate are shown in Figure 2. Hormone receptors are precipitated from the supernatant cytosols by the addition of protamine sulfate [9]. [^{3}H]-estradiol or [^{3}H]-progesterone is then added to the precipitated receptor proteins. The unbound radioactive hormone is washed from the precipitated receptor preparation and bound hormone is estimated by liquid scintillation counting.

We demonstrated a high-affinity (kd = 4.0×10^{-9} M) receptor for [^{3}H] estradiol in our patient's hepatic neoplasm (Fig. 2). The data are replotted in the inset using the Scatchard technique [15]. The straight line obtained ($r = -0.987$) is consitent with estradiol binding to a single class of receptor sites of uniform affinity. From the x-intercept of this plot we estimate that 47.9 femtomoles of estradiol were bound per mg of cytoplasmic protein. The assay conditions employed allow for receptor binding to reach equilibrium. However, metabolism or conjugation of the labeled estradiol by the cytosol may occur. If this took place to any significant extent the net effect would be to lower the apparent affinity of the receptor but not the x-intercept. The binding technique we employ [9] is not interfered with by contamination with serum binding components such as sex steroid binding globulin. This is realized in two ways. First, the protamine sulfate precipitation technique separates estrogen receptor from sex steroid-binding globulin. Secondly, unlabeled 5-α-dihydrotestosterone added to both

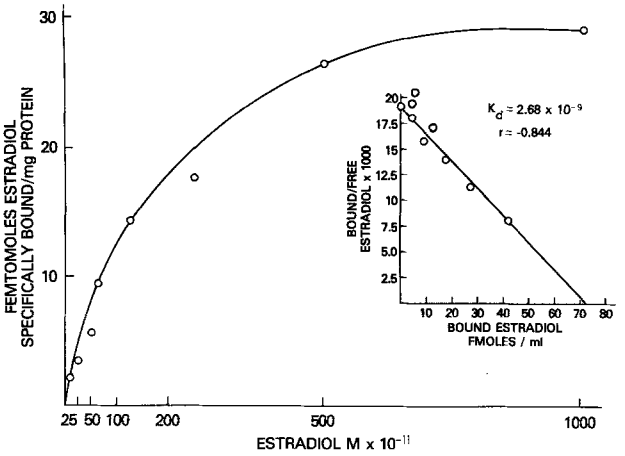

Fig. 2. Specific binding of ^{3}H-17B-estradiol to receptor sites in a 100,000 *g* supernatant fraction prepared from hepatic nodular hyperplasia described in text. Scatchard plot of same binding data is shown in inset. Our methods for receptor analysis have been described elsewhere [10]

competed and uncompeted tubes competes with [^{3}H] estradiol for binding to sex steroid-binding globulin but not estrogen receptor [9]. Progesterone receptor levels were also assessed by means of a competitive binding technique [10]. A specific progesterone receptor was found, (kd = 2.8×10^{-9} M, $r = 0.896$) with a binding capacity of 146 femtomoles/mg protein. We have presented elsewhere [10] our validation of this receptor method for progesterone receptor. It is specific in that it is not interfered with by [^{3}H]-progesterone binding to either corticosteroid binding globulin (CBG) or glucocorticoid receptor. In brief, this is achieved by adding 10^{-6} M unlabeled cortisol to both competed and uncompeted tubes in the progesterone assay. Cortisol competes with [^{3}H]-progesterone binding to CBG and glucocorticoid receptor. The Kd obtained is similar to that found in human breast cancer in tissue culture [8].

Discussion

Focal nodular hyperplasia [14] is an hepatic neoplasm that has been associated with the ingestion of estrogenic hormones, and our patient with this lesion had had two episodes of exposure to exogenous hormonal therapy. Twenty-three years prior to admission she was treated weekly for 1 year with the synthetic estrogenic hormone, diethylstilbesterol. Five years prior to admission she was exposed to 3 monthly cycles of therapy with an oral contraceptive containing the progestational agent norethynodrel and the estrogen mestranol. It should be noted that the second exposure to estrogenic material occurred after the patient first became aware of a right upper quadrant mass. Although we have demonstrated high-affinity estrogen binding protein in our patient's neoplasm, this cannot be taken as evidence that her previous estrogen therapy is the etiology of her focal nodular hyperplasia of the liver. It is known, however, that estrogen is a potent stimulant of hepatic cell proliferation. Aldercreutz and Tenhunen have reviewed experimental data demonstrating that low doses of estradiol stimulate hepatic regeneration in the rat [1].

Normal liver may bind a finite amount of estrogen, and Eisenfeld and colleagues have recently shown that in female rats there are 8.7 femtomoles estradiol bound per mg of cytoplasmic protein [4]. This value is only a small fraction of that found in other rat target tissues, such as uterus [7]. One of us (M.L.) has performed estrogen receptor assays on normal liver from three patients. The results revealed 3.0, 11.4, and 13.6 femtomoles per mg. The first and last values were obtained from postmenopausal females; the middle value from a premenopausal female. It can be seen that these values range from 6% to less than 33% of the 47.9 femtomoles estradiol bound by our patient's tumor. It would have been of great interest to measure hormone receptor levels in normal liver from this patient; however, she did not consent to percutaneous liver biopsy.

An additional point in favor of possible estrogen receptor-mediated stimulation of this tumor derives from our detection of a specific progesterone receptor as well as an estrogen receptor. Estrogen has been shown to regulate progesterone receptor levels in several tissues [13], and such a mechanism has been suggested in human breast cancers. The presence of a progesterone receptor has been tentatively linked to estrogen responsiveness in human breast cancer [6].

The demonstration of a high-affinity estrogen receptor in an estrogen-associated hepatic neoplasm is consistent with an hypothesis that a small proportion of normal hepatic cells have relatively high concentrations of cytosol estrogen receptors, which render them particulary susceptible to stimulation to a neoplastic state by estrogen. Prolonged therapy with exogenous estrogenic hormone may then lead to clinically apparent hepatic tumors, which contain high levels of cytosol estrogen receptors. The testing of this hypothesis requires conclusive definition of the role of cytosol estrogen receptors in hepatic neoplasia. This can only by achieved by a systematic study of patients with these tumors. We would urge the performance of estrogen and progesterone receptor assays on tumor and normal liver whenever patients with potentially estrogen-related hepatic neoplasia undergo surgical exploration or percutaneous liver biopsy. Only in this way will it be possible to define the relationship between estrogen receptor levels and the presence of these tumors.

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