

REVIEW ARTICLE

HEPATOCELLULAR CARCINOMA AND SEX HORMONES

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The liver is morphologically and functionally modulated by sex hormones. Long-term use of oral contraceptives and androgenic steroids can induce benign and malignant hepatocellular tumors. Hepatocellular carcinoma (HCC) is more prevalent in men than in women. The role of sex hormones and their receptors in the development of HCC was reviewed. Some HCCs may be androgen dependent but others may be estrogen or even both dependent. Further studies are mandatory in order to utilize such characteristics of HCC for an effective prophylaxis and therapy of this tumor.

KEY WORDS: Hepatocellular carcinoma, sex hormone, androgen, estrogen, progesterone, receptor

Cirrhosis of the liver is frequently associated with hepatocellular carcinoma (HCC). It is well known that cirrhotic male patients, particularly those with alcoholic cirrhosis, have impaired function of the hypothalamic-pituitary-gonadal axis¹. All over the world, HCC is more prevalent in men than in women. This is especially true in patients with cirrhosis and in the areas where HCC is common. Such male predominance of HCC may be related to sex hormone imbalance². On the other hand, it is undeniable that long-term use of oral contraceptives induces not only benign but malignant tumors of the liver. Also, androgenic anabolic steroids, especially 17-alkyl-substituted steroids, seem to be related to the development of liver neoplasms including HCC. These facts raise the question; what is the genuine role of sex hormones in hepatocarcinogenesis in experimental animals and particularly in humans?

Liver Tumors and Sex Steroid Administration

The effect of sex hormones on liver function and morphologic features has been pointed out over the past two decades. It is generally accepted that the liver is one of the target organs for estrogens. Since the first report by Baum *et al.*³, a possible correlation of estrogens with the development of hepatic neoplasms has been suggested by numerous reports of oral contraceptive-associated focal nodular hyperplasia (FNH) or liver cell adenoma. Malignant transformation has also been reported in such patients. In addition, withdrawal of oral contraceptives occasionally induces regression of the drug induced hepatic tumors⁴⁻⁶. According to the

recent articles by Neuberger *et al.* and Forman *et al.*⁸, the use of contraceptive pills for longer than 8 years is associated with a 20-fold increased risk of HCC.

On the other hand, the administration of exogenous androgens, particularly 17- α -alkylated androgens, causes various hepatic lesions such as dilation of biliary canaliculi, loss of canalicular villi, peliosis hepatis, adenoma, and HCC⁹. Such changes occur less frequently with non-17- α -alkylated androgens. It is noteworthy that synthetic estrogen and progestogen used for pills have a similar chemical structure to androgenic steroids.

Although most carcinogens can be shown to be mutagenic *in vitro*, this has not been demonstrated for estrogens¹⁰. The Committee on Safety of Medicine (London) reported a tumorigenic action of synthetic estrogens in male rats but not in female rats¹¹. Reznik-Schuller¹² found the development of liver tumors (hepatocellular adenomas, carcinomas, and cholangiocarcinoma) in 29% of male European hamsters treated with diethylstilbestrol (DES). This seems to be the first report showing that estrogens alone can be carcinogenic. Other authors have suggested that estrogens promote the effect of carcinogens¹³⁻¹⁵. There are several groups in Japan who have extensively studied this problem. Sumi *et al.*¹⁶ initially found a synergistic effect of DES and carcinogens on the development of liver tumors in male WF rats. Later, they have found that DES itself has a direct carcinogenic effect on the liver and that this effect is not mediated by prolactin¹⁷. Higashi *et al.*¹⁸ in 1980 reported that synthetic estrogen and progestogen could induce HCC in female Wistar rats.

Despite a considerable number of reports on androgenic steroid-induced hepatic tumors, there is limited evidence for a direct etiologic relationship between administration of the drugs and HCC in humans¹⁹. Many years ago, Morris *et al.*²⁰ found that in a hepatic carcinogenesis model in rats with 2-diacetylaminofluorene HCC occurs more frequently in intact males and castrated females treated with testosterone than in intact females, castrated males, castrated females, and castrated males treated with DES. Whether or not androgenic steroids are carcinogenic remains to be elucidated.

Sex Hormone Receptors in HCC

It is theoretically assumed that the tumorigenic effect of estrogens may be through an estrogen receptor (ER). The presence of ER in human liver was first found by Duffy and Duffy²¹ in 1978. Friedman *et al.*²² estimated ER levels in human HCCs. The titer of ER in HCC taken from 5 male adults ranged from 0.9 to 6.4 fmol/mg cytosol protein. We have measured ER of HCC in 30 adult patients²³. ERs were present in 12 (40%) tumors. Since 29 were male in that study, we next assayed the receptor in 19 women²⁴. ERs were detected in 7 of them (37%). Ohnishi *et al.*²⁵ found the receptor in only one of 6 HCCs. At present, it is not well known if the presence of ER in HCC is really related to the development and growth of the tumor in humans. Li and Li²⁶ suggested a possibly important role of ER in hepatocarcinogenesis since a higher value of both cytosolic and nuclear ERs in hamster livers was observed when the regimen of DES and α -naphthoflavon was made more tumorigenic. Considering this and previously described experimental and clinical studies, ERs in the liver or HCC may also play a role in human hepatic carcinogenesis.

It has long been believed that human liver is not a target organ for androgen. In

1985, we first observed that normal human liver possesses androgen receptors (AR)²⁷. Immediately after our report, Bannister *et al.*²⁸ presented a similar observation. Iqbal *et al.*²⁹ were the first to find ARs in human HCC. We have also shown that ARs are present in 74% of male and 37% of female HCCs^{24,27}. In addition, the titers were significantly higher in HCC than in the surrounding liver parenchyma. A very similar result was presented by Ohnishi *et al.*²⁵. We thereafter investigated, with positive results, if extrinsically given testosterone were taken up by AR in HCC using autoradiographic techniques. All over the world, HCC is more prevalent in males than it is in females³¹. This is especially true in patients with liver cirrhosis³². Male predominance is usually explained by the fact that alcoholism, chronic liver disease, and in particular chronic hepatitis B infection are more prevalent among males. In the light of our serial receptor studies, however, we have suggested that some, if not all, human HCC may be androgen-dependent. Such a speculation is supported by our clinical results which will be described in the next section.

Several investigative groups have shown that high titers of progesterone receptor (PgR) are present in FNH and liver cell adenoma whether or not they are induced by oral contraceptives³³⁻³⁵. Demenes *et al.*³⁶ found a similar result in hepatoblastoma. However, very little is known of whether PgR is present in HCC or not^{22,34}. We estimated PgR as well as AR and ER in 22 consecutive patients with HCC³⁷. PgR was considered to exist when the titer was higher than 1.0 fmol/mg of protein. The receptor was detected in the cytosol of HCC in 4 patients (18.2%), and the titers were low ranging from 1.1 to 3.0 fmol/mg of protein. Thus, high concentrations of PgR exist in benign liver tumors or hepatoblastoma, but not in HCC. At present, it is not known why most HCCs are negative for PgR or the titer is so low if present at all.

Operative Results of HCC

In our series of 107 radical hepatic resections, the 1-, 3-, and 5-year survival rates in male and female patients were 78% and 70%, 45% and 52%, and 19% and 52%, respectively³⁸. The difference was significant 47 months after operation. The clinicopathologic background was not different between men and women. Thereafter, we have analyzed the recurrence and survival rates between the patients with AR-negative and AR-positive HCCs³⁹. The recurrence rates were 33.8% in the former and 67.9% in the latter group. Also, the 5-year survival rate was significantly better in the patients with AR-negative HCCs (62.2%) than in the patients with the positive tumors (17.3%). On the other hand, the presence or absence of ER in HCC did not influence the long term results⁴⁰.

Hormone Therapy of HCC

Friedman *et al.*²² first tried hormone therapy for HCC. Since in hormone-responsive tissue such as breast or endometrial carcinoma that contains ERs, administration of progestins results in decreased ER titers and often causes tumor regression, they performed progestin therapy in 5 patients with HCC. Tumor regression was observed in two of them. We have treated 9 patients with nonresectable HCC with tamoxifen, but no apparent tumor response was found (unpublished data). As tamoxifen inhibits the development of hyperplastic nodules in the

liver of experimental animals⁴¹, anti-estrogen therapy may be effective in selected patients with HCC. However, we probably cannot expect so much solely by anti-estrogen therapy in the treatment of far advanced HCCs. Based upon the fact that human HCCs contain AR, Forbes *et al.*⁴² performed anti-androgen therapy with cyproterone acetate. They have found objective responses in 5 of 25 patients with advanced HCCs. We have also obtained a similar but a little worse result in patients with nonresectable or recurrent HCCs (unpublished data). Recently, Bannister *et al.*⁴³ and more recently Ostrowski *et al.*⁴⁴ reported that the development of HCC is strongly related to the increased hepatic AR concentrations in diethylnitrosamine induced hepatocarcinogenesis in rats. Using human HCC cell lines, Ohnishi *et al.*⁴⁵ have shown that the growth of AR positive cells was enhanced by dihydrotestosterone, and was inhibited by adding cyproterone acetate.

Future Perspective

Except for oral contraceptive-associated HCCs which may be highly estrogen-dependent, human HCCs seem to be more androgen-dependent. Male predominance, the receptor profiles, and the clinical evidence may support this speculation. Routine receptor assays of surgically removed specimens will be useful for further elucidation of this unsolved problem. Based upon the receptor result in each individual, appropriate hormone therapy which may be anti-androgenic, anti-estrogenic, or both will be possible. The poor survival in men after liver resection could theoretically be improved by adjuvant anti-androgen therapy. It may be possible to improve the survival rate of those with nonresectable HCCs by combining hormone therapy with conventional chemo- or immuno-chemotherapy. If hormonal manipulation can prevent the development of HCC in high-risk patients will be a matter for future work.

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