Sex hormones and liver cancer

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignancy in the world and it usually occurs in individuals with chronic liver disease. The neoplasm is predominant in the male gender, where it is characterized also by a worst prognosis than in females. The pathogenesis of HCC is obscure. Because of its striking male predominance, androgens have been investigated as potential factors able to induce or at least promote hepatic carcinogenesis; this hypothesis has been also supported by the ability of androgens of inducing liver neoplasms in experimental models. On the other hand, due to the fact that HCC occurs predominantly in male cirrhotics who present a characteristic hormone imbalance with a relative hyperestrogenic state, the potential role of estrogen in liver cancer has been studied as well. In this paper, the potential role of sex hormones in liver carcinogenesis has been reviewed.

Keywords: Liver cancer; Estrogen; Androgen; Hormone receptor; Carcinogenesis

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world. The incidence of HCC has increased in the last decades worldwide: a number between 500 000 and 1 000 000 of new cases of HCC are reported every year, and HCC is responsible for about 750 000 deaths per year (International Agency for Research on Cancer, 1997; Ikeda et al., 1993; Fattovich et al., 1997). HCC usually occurs in individuals with chronic liver disease: in fact, the risk of developing HCC per year in cirrhosis ranges from 2 to 8%, depending on the different etiologies of the underlying cirrhosis (International Agency for Research on Cancer, 1997; Ikeda et al., 1993; Fattovich et al., 1997).

The incidence of HCC varies considerably in the different areas of the world, being higher in the Eastern Asia and Sub-Saharan Africa and low in North America including USA (International Agency for Research on Cancer, 1997; Ikeda et al., 1993; Fattovich et al., 1997). The incidence in Europe is on the intermediate side, 4.0 to 12.5/100 000 and is much higher in Southern Europe, where in some areas it may reach 20 cases per 100 000 individuals (International Agency for Research on Cancer, 1997; Ikeda et al., 1993; Fattovich et al., 1997).

The pathogenesis of HCC is still poorly understood: in fact, a multi-step sequence of events leading from normal hepatic tissue to the development of cancer, possibly through some intermediate pre-cancerous lesions, has not been identified yet. What we know is that some factors are associated with an increased risk of development of the cancer. Table 1 reports the most important risk factors for HCC: as one can see, the male gender is among them. HCC, in fact, has a striking increased predominance in males, with a male to female ratio ranging from 2 to 11 to 1 in most published series (El-Serag et al., 1977). Not only males develop HCC more often than females, but once they develop the cancer, they also die more easier than females: in fact, the prognosis for the disease is more benign in females than in males and women have a better survival and a reduced recurrence of the disease after treatment (El-Serag et al., 1977).

From all these evidences, the interest of some researchers has been focused since the 80’s on the possible importance of sex hormones in determining such preference for the male gender. To make things more complicated, it needs to be remembered also the unique hormonal alteration that characterizes the male individual who develops HCC. In fact, as it has been said before, HCC occurs more often in males with chronic liver disease. Being males, these individuals have been under the constant influence of androgens for their
life, but due to the presence of the underlying liver disease they also present a characteristic alteration of the hormonal milieu, with a so called ‘feminization’ of their phenotype due to a relative hyperestrogenic state. As a result, both the presence of male sex hormones and the effect of the cirrhosis-induced feminization have been blamed as responsible, at least in part, for the development of HCC.

2. HCC: the role of androgens

Androgens have been known for long time to be associated with an increased incidence of liver neoplasms: back in 1952, Agnew et al. reported the spontaneous occurrence of hepatic tumors in male rodents and the increased incidence of such tumors in different strains of mice chronically exposed to androgens (Agnew and Gardner, 1952). Subsequent studies have revealed that male rodents are more susceptible to hepatocarcinogenesis not only chemically induced, but also in experimental models of chronic viral infection (Firminger and Reuber, 1961; Kemp et al., 1989): for example, male transgenic mice for HBV or HCV develop HCC more often than female (Kim et al., 1991; Moriya et al., 1998). These findings have been confirmed both by in vitro studies, where the growth and proliferation of hepatic normal or tumor cell line has been shown to be enhanced by dehydrotestosterone (DHT) and testosterone, and by the clinical practice, since the use of androgenic steroids is associated with an increased risk of developing liver neoplasms including HCC (Mokrohisky et al., 1977; Farrell et al., 1975; Johnson et al., 1972; Westaby et al., 1947).

Like the other steroid hormones, androgens exert their effects through the activation of specific hormone receptors. In fact, receptors specifically activated by testosterone and DHT have been identified on the cytoplasm and the nucleus of the hepatocyte. These receptors are present in the normal liver tissue from both male and female mammalians, but their expression and activation is reported to be increased in the tumor tissue and in the surrounding liver tissue of individuals with HCC (Ohnishi et al., 1986; Nagasue et al., 1985a, 1989; Eagon et al., 1991; Ostrowski et al., 1988; Nagasue et al., 1995). Moreover, the expression and activation of the same receptors is reported to be greatly increased in the liver tissue of male and female rodents during chemically-induced liver carcinogenesis (Ohnishi et al., 1986; Nagasue et al., 1985a, 1989; Eagon et al., 1991; Ostrowski et al., 1988; Nagasue et al., 1995). The presence of androgen receptors (AR) has also been associated to an increased risk of tumor recurrence and to a reduced survival after hepatic resection for HCC. According to a study of Nagasue et al., individuals who had AR negative tumors showed a survival of 55% at 5 years after surgery, while those with AR positive tumors had a survival rate of 0% (Nagasue et al., 1995).

The hepatic effect of androgens is clearly receptor-mediated, since their effect on tumor growth is inhibited by the concomitant presence of anti-androgen substances that specifically block the AR. In fact, the castration or the use of anti-androgen treatment protect male rodents from tumor development (Vesselinovitch and Mihailovich, 1967; Toh, 1981; Vesselinovitch et al., 1980). Moreover, castrated female rodents receiving testosterone have a susceptibility to tumor development similar to that of intact males; this susceptibility is easily reverted by the use of antiandrogenic drugs (Matsuura et al., 1994).

Recent studies have focused their attention on the characteristics of the AR gene. The AR gene is located on the long arm of the X gene. Its transactivation domain is characterized by the presence of CAG repeats encoding for glutamine residues in the N-terminal domain of the AR protein (Giovannucci et al., 1997). The number of CAG repeats in the transactivation domain of the AR gene appears to control the AR gene expression: an increased length of the glutamine residues region inhibits the interaction of AR with activators and co-activators (Giovannucci et al., 1997). In the prostate tissue, AR genes with fewer CAG repeats are associated with an increased risk of prostate cancer (Giovannucci et al., 1997; Chamberlain et al., 1994).

Yu et al. have published a study in which the sex hormone balance and the AR gene structure was assessed in a large cohort of HBV positive individuals followed for 7 to 11 years (Yu et al., 2000). Blood samples were collected at the time of enrollment for the determination of testosterone levels and for the assessment of the CAG repeats length on the AR gene. As result, the authors showed that testosterone levels were higher in patients who developed HCC and that livers with an AR gene with 20 or fewer CAG repeats had a greater risk of cancer than those with >24 repeats. As a consequence, individuals with AR gene with <20 repeats and high testosterone levels have a four fold increase in HCC as compared with those with >24 repeats and lower testosterone levels.

<table>
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<th>Risks factors for HCC</th>
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<td>HBV or HCV infection</td>
<td>Afatoxin exposure</td>
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<tr>
<td>Cirrhosis</td>
<td>Alcohol abuse</td>
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<tr>
<td>Metabolic liver diseases</td>
<td>Carcinogen exposure</td>
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<td>Steroids</td>
<td>Male gender</td>
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Table 1
On the same line of research, Tanaka et al. published a follow-up study on 46 male individuals with HCV-related cirrhosis (Tanaka et al., 2000). Serum testosterone, free testosterone, estradiol and SHBG were assessed at the time of enrollment in the study and the patients were followed for an average of 5.1 years. The results of the study showed that the serum testosterone/estradiol ratio and the testosterone levels are predictors for HCC development at an univariate analysis; moreover, free testosterone levels are associated with an increased risk for HCC at the multivariate analysis. The conclusion of the study was that elevated serum testosterone levels together with decreased estrogens may promote the development of HCC in cirrhosis.

Considering all this amount of data, one could conclude that the liver is an androgen-sensible organ with a biological behavior similar to that of the prostate; thus, HCC might be an hormone-sensible tumor like prostate cancer and therefore should respond to an anti-androgen treatment. Several studies have utilized different anti-androgenic compounds in an attempt of treating or at least reducing the progression of liver cancer. However, the results have been quite disappointing so far, as most of the published studies showed a complete lack of effect of this therapeutic approach. Two large studies have been published recently: in the first one, Chao et al. assessed, in a phase I study, the clinical activity and toxicity of flutamide, an anti-androgenic compound, in 32 patients with unresectable HCC (Chao et al., 1996). The patients received flutamide 750 mg/day for 8 weeks; at the end of treatment, no complete or partial responses were observed. The authors concluded that flutamide is not effective in HCC and that HCC may not be an androgen-responsive tumor. In the second study, Grimaldi et al. reported the experience of a multicentric double-blind trial with 244 patients with unresectable HCC randomized to receive different regimens of anti-androgens or placebo (Grimaldi et al., 1998). No significant difference among the groups was reported at the end of the study and the anti-androgen treatment was considered again as not effective in HCC.

3. HCC: the role of estrogens

Together with the receptors for androgens, the normal liver tissue form male and female mammals have high-affinity, low-capacity, saturable and specific estrogen receptors (Johnson, 1984; Friedman et al., 1982; Eisenfeld and Aten, 1987; Carson-Jurica et al., 1990). Their importance in the normal liver physiology is not clear, but it has been shown that estrogens play an important role in the control of liver cell proliferation (Francavilla et al., 1993). In fact, estrogens are involved in the regulation of hepatocyte proliferation: a ‘feminization’ of the hepatic microenvironment occurs after partial hepatectomy in rats and humans with an increased in the estrogens level and a concomitant reduction of testosterone levels (Francavilla et al., 1993). Moreover, the hepatic ERs increase and are actively translocated to the nucleus after partial hepatectomy in humans and rats (Fisher et al., 1984; Francavilla et al., 1984, 1989). Anti-estrogens, like tamoxifen, reduce the levels of both cytosolic and nuclear ER and inhibit hepatocyte proliferation following partial hepatectomy.

Chronic liver diseases determine a specific alteration of the sex hormones balance that is more evident especially in the male individual. As it has been remembered before, cirrhotics have a unique alteration of their endocrine milieu characterized by a hormone imbalance with an absolute or relative hyperestrogenic state manifested clinically by the occurrence of a ‘feminized’ phenotypic appearance (Farinati et al., 1995; Nagasue et al., 1985b; Montalto et al., 1997; Guechot et al., 1988). This feminization is the result of a direct effect on gonads by toxic agents (i.e. alcohol), altered hormone metabolism due to chronic liver disease, and failure of the hypothalamus–pituitary–gonadal axis. The activity of cytosolic ERs is also increased in liver diseases in males, enhancing the responsivity of male liver to estrogens (Farinati et al., 1995; Nagasue et al., 1985b; Montalto et al., 1997; Guechot et al., 1988). Moreover, the serum estradiol to testosterone ratio is higher in individuals with HCC and cirrhosis than in normal individuals or individuals with cirrhosis alone (Farinati et al., 1995). It is not clear if this may be a pathogenic factor in HCC or just an epiphenomenon.

The estrogens are well known carcinogenic agents in estrogen-responsive tissues (breast, uterus). In liver, experimental models have shown that estrogens act as tumor promoters and may induce hepatocarcinogenesis in hamsters and mice; estrogens may induce also the formation of free radical-mediated DNA and RNA adducts potentially mutagenic (Yager and Yager, 1980). In humans, the chronic use of estrogens is associated with increased risk of developing liver neoplasms such as benign nodular hyperplasia and hepatic adenoma (Baum et al., 1973; Davis et al., 1975; Christopherson et al., 1975). Estrogens have also been described as putative agent of HCC in humans and nuclear ERs level in neoplastic liver is higher than in normal tissue (Christopherson et al., 1975).

Considering all these premises, one can reach the opposite conclusion that the liver is a hormone-responsive organ like breast tissue; HCC could therefore be an estrogen-dependent cancer like breast cancer and the use of anti-estrogen drugs should control the growth of this tumor.

Several studies have used tamoxifen for the treatment of HCC and the results appeared to be initially
encouraging. Tamoxifen (TMX) is an anti-estrogen drug used for the treatment of breast cancer. TMX has several other biologic activities that may have relevance in cancer treatment: inhibition of PKC, calmodulin, TGF-α and TGF-β1 induction; antagonism of estrogen binding to the erbB-2 oncogene; and activation of NK-mediated cytoxicity. Some of these may be responsible for the reported effects of TMX on various cancers (lymphoma, gliomas, melanoma). Most of the studies published in the early 90’s (all of them based on small number of patients) reported a reduced tumor growth with prolonged survival in individuals treated with tamoxifen as compared with untreated controls. The results seemed to be so reliable and consistent that a meta-analysis published in 1996 on the palliative treatment for HCC indicated tamoxifen as one of the few therapeutic approaches with a clear and significant beneficial effect (Simonetti et al., 1997). However, such conclusion was later contradicted by the results of two large trials utilizing tamoxifen. An Italian group published a paper on Lancet reporting the results of a multicentric trial with 496 pts with HCC at any stage randomized in two matched group to receive tamoxifen 40 mg/day or placebo (CLIP Group, 1998). The median survival was 15 and 16 months, respectively, and the conclusion was that tamoxifen is not effective in HCC treatment. Same results from Liu et al.: 119 patients with unresectable HCC were randomized in two matched groups to receive tamoxifen 30 mg/day or placebo (Liu et al., 2000). The median survival was 44 and 41 days, respectively, and no relation between better survival and presence of ER receptors was found.

4. Conclusions

Experimental and clinical data have shown that both estrogens and androgens have important effects in controlling the replication rate of hepatic cells. Both estrogens and androgens may also have effect on inducing or at least promoting the growth of liver tumors, including HCC. However, the disappointing results obtained by anti-estrogen and anti-androgen treatments may suggest that either the suppression of their effect, once the tumor has developed, has probably no clinical relevance on the progression of the disease or that ‘clinically’ HCC is not a sex hormones responsive tumor. The recent finding of the presence of varied estrogen receptors in HCC, like in breast cancer, may open a different way for the interpretation of these data.

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