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Advances in Brief

Type of Estrogen Receptor Determines Response to Antiestrogen Therapy[1]

Erica Villa,[2] Aisha Dugani, Emanuela Fantoni, Lorenzo Camellini, Paola Buttafoco, Antonella Grottola, Guglielmo Pompei, Mario De Santis, Alberto Ferrari, and Federico Manenti

Division of Gastroenterology, Department of Internal Medicine, University of Modena, Via del Pozzo 71, 41100 Modena [E. V., A. D., E. F., L. C., P. B., A. G., A. F., F. M.], Institute of Radiology, Ospedale Civile, 41058 Vignola [G. P.], and Institute of Radiology, Azienda Policlinico, 41100 Modena [M. D. S.], Italy

Abstract

Failure of tamoxifen treatment for unresectable hepatocellular carcinomas (HCCs) might be caused by variant estrogen receptors (ERs) in some of these tumors. We therefore planned a study in which antihormonal therapy was done with 80 mg/day tamoxifen or 160 mg/day megestrol according to the presence of wild-type or exon 5-deleted variant ER transcripts.

Growth rate (evaluated by MRI) of HCCs characterized by variant ER transcripts was 4 times more rapid than that of HCCs with wild-type ERs. Tumor volume in all patients with wild-type ERs was halved after 9 months of tamoxifen treatment, whereas megestrol in patients with variant ERs only slowed down tumor growth.

Choosing antihormonal treatment according to the presence of wild-type or variant ERs in the tumor definitely improves the response rate to tamoxifen; in patients with tumors bearing variant ERs, megestrol causes only a temporary inhibition of tumor growth.

Introduction

Unresectable HCC[3] is a frequent occurrence, despite the widespread use of ultrasonographic screening of cirrhotic patients for the early detection of neoplastic lesions. This has caused development of a variety of palliative procedures (embolization, chemoembolization, or alcohol injection), which, however, have not caused a significant improvement of these situations (1, 2). The presence of ERs in the liver (3, 4) and the supposed causal relationship between sex steroids and liver tumors (5, 6) have suggested the possibility of using tamoxifen as a palliative treatment in inoperable HCC. Although some patients have shown reduction of tumor mass and decrease of AFP, most patients have failed to show any response (7–9). The presence in a substantial proportion of HCCs of a vER that is modified in the hormone-binding domain and is unable to bind estradiol (10) may explain tamoxifen failure because one of the main mechanisms of action of this drug is occupation of receptor site (11). In HCCs characterized by a vER, an alternative drug, acting at the postreceptor level, would have a better chance of success. We have therefore planned a pilot study in which antihormonal therapy was decided on the basis of the type of ER transcript present in the liver. Patients with wtER were treated with tamoxifen, whereas those with vERs were treated with megestrol, a progestin drug that has a strong antiestrogen action, independent of receptor binding.

Materials and Methods

Eight cirrhotic patients [three females (mean age, 58 ± 8 years) and five males (mean age, 57 ± 13 years)] with a diagnosis of unresectable, multifocal primary HCC underwent ultrasound-guided biopsy of the neoplastic nodules and of the surrounding tissue. Six patients were class A, one was class B, and one was class C according to Child-Pugh classification.

RNA was extracted, reverse transcribed, and amplified as described previously (10). The amplified fragment corresponding to the wtER was 438 bp, and the vER was 296 bp.

Four patients with wtERs were assigned to 80 mg/day tamoxifen, whereas four patients with vERs were assigned to 160 mg/day megestrol. No special biochemical control was necessary during therapy apart from evaluation of hypercoagulative signs in patients on tamoxifen.

To evaluate tumor growth, patients underwent MRI at enrollment in the study (t_0) and after 3 months without therapy. MRI was repeated 3 (t_6) and 9 (t_{12}) months after starting therapy. At each time point, number and diameter(s) of each nodule were recorded. Tumor volume was estimated to be $4\pi r^3/3$, where r is the radius of the tumor measured at MRI. Total volume of neoplastic mass was obtained summing the volume of each nodule. In case of nonspherical tumors, the volume was calculated according to the formula:

$$V = \frac{4}{3}\pi\sqrt{(a/2 \times b/2)^3}$$

where a and b are the largest and the smallest diameter of the tumor. Growth rate of HCC was expressed as tumor volume doubling time ($t \times 2$) according to the formula:

$$\frac{(T_2 - T_1) \times \log_e 2}{(\log_e V_2 - \log_e V_1)}$$

where $(t_2 - t_1)$ is the period of time (in days) between two consecutive observations, and V_2 and V_1 are the volume of the tumor evaluated at the last and first examination, respectively. The formula is derived from previously published papers and is based on the concept of an exponential growth of the tumor (12, 13).

The comparison between data obtained at baseline and at month 3 and between those at month 6 or 12 and month 3 permitted us to evaluate the rate of growth without and with therapy.

Statistical analysis was performed with the paired or unpaired Student's t test when appropriate. A P value <0.05 was considered significant.

The study was approved by the Institutional Ethical Committee of the University of Modena; patients gave informed written consent before enrollment in the study.

Results

All patients have completed at least 1 year of follow-up. Both treatments were well tolerated; none of the patients dropped out. One patient in the megestrol group died of spontaneous bacterial peritonitis 13 months after diagnosis. The other patients in the megestrol-treated group reported a remarkable increase in body weight related with a significantly increased appetite and feeling of well-being.

Four patients (three males, one female) were characterized by wtER both in the tumor tissue and in the cirrhotic tissue surrounding the tumor; four patients (two males, two females) displayed in the tumor

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[2] To whom requests for reprints should be addressed. Phone: 39-59-422-250; Fax: 39-59-424-363; E-mail: ericavil@unimo.it.

[3] The abbreviations used are: ER, estrogen receptor; wtER, wild-type ER; vER, variant ER; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging; AFP, α-fetoprotein.

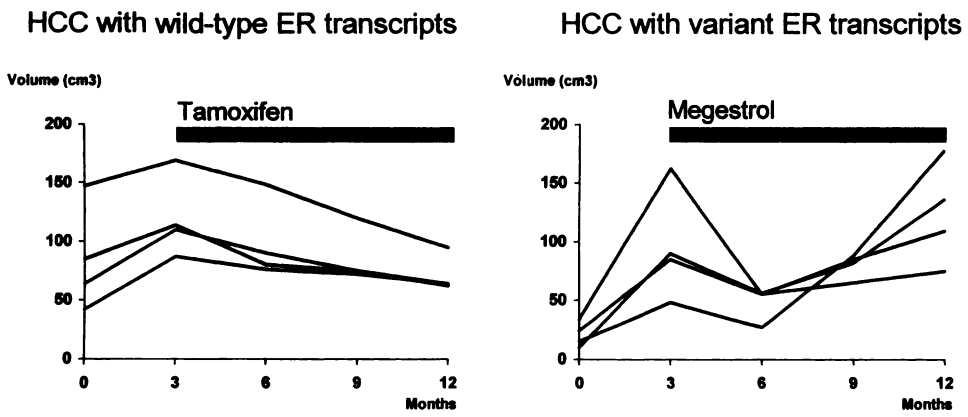

Fig. 1. Modifications of liver tumor volume in patients with unresectable HCC undergoing antihormonal therapy with tamoxifen or megestrol, divided according to ER status.

tissue only the vER, whereas in the peritumoral tissue, a mixture of wtER and vER transcripts was present.

Tumors with vERs were characterized by a much higher rate of growth than tumors with wtER: in the initial 3 months of observation without therapy, the volume of neoplastic tissue in the patients with vERs increased 5-fold, whereas in patients with wtER, the growth did not exceed 1.4 times the baseline volume.

Tamoxifen produced a significant decrease in the rate of growth of wtER-bearing tumors in all patients treated: the t_{12}/t_3 ratio below 1 at the end of the 9 months of therapy indicated that neoplastic mass was indeed decreased (Fig. 1). Corresponding to this decrease was the decrease of AFP in the three patients in whom AFP was abnormal (AFP at 3 months *versus* 1 year, 520 ± 250 *versus* 230 ± 120 ng/ml).

In patients with vERs, megestrol produced a significant slow-down of tumor growth: in one patient, tumor growth was greatly inhibited, and in the other three patients, after an initial decrease in the first 3 months of therapy, it began to increase again during follow-up (Fig. 1). AFP was abnormal in all patients; values decreased from 1397 ± 180 at 3 months to 930 ± 760 ng/ml at 1 year.

Tumor volume doubling time was significantly shorter in HCCs with vERs in comparison with those with wtERs. In the latter patients, regression of tumor volume with tamoxifen correlated with the significantly longer doubling time. In patients with vERs, doubling time was slowed down, although not significantly (Table 1).

In none of the tamoxifen-treated patients was there occurrence of new focal lesions, whereas in two megestrol-treated patients, several new satellite lesions with a diameter of less than 1 cm were present at 1 year.

Discussion

A remarkably different rate of growth for individual HCC has been often described by many authors (13, 14); we have shown that this difference may be explained by the molecular characteristics of the tumor.

HCCs displaying vERs show a growth rate 4 times higher than that of HCCs with normal ERs. This finding is in agreement with the data reported in breast cancer, in which the occurrence of vER usually coincides with loss of hormonal dependence and acceleration of neoplastic growth (15). Our findings are further reinforced by the observation during follow-up that in HCCs with vERs, not only was the increase in volume of the neoplastic nodules more aggressive but also there was a higher occurrence of new lesions. This fact, together with the impossibility of tamoxifen of exerting an effective blockage of the vERs (11), might explain the disappointing results of the trials performed thus far (7–9). It is therefore not surprising to observe a striking improvement of the overall response rate to tamoxifen when this drug is administered only to patients with wtERs: these are in fact the patients in whom the occupation of the receptor by tamoxifen, blocking transcriptional activation of estrogen-responsive genes, would have the best chance of being effective. Many authors have suggested that tamoxifen may have different modes of action apart from blockage of the ERs, namely inhibition of protein kinase C (16), inhibition of protein tyrosine phosphorylation at the level of src signal transducers (17), inhibition of calmodulin, and interaction with growth factors (18). Our results emphasize, however, that the receptor blockage is an extremely important, and perhaps the most important, antineoplastic mechanism: in all patients with wtER, we have obtained a clear-cut inhibition of growth, with halving of the neoplastic mass during the 9 months of treatment.

The results with megestrol in tumors with vERs have been less impressive; however, in all patients, there has been a remarkable slow-down of tumoral growth rate (as shown also by AFP decrease). Taking into account the fact that these tumors showed a much higher spontaneous growth in the baseline months without therapy, the overall result can be considered promising. Megestrol has been already tested in the past for treatment of inoperable HCCs (19). Friedman *et al.* (19) showed tumor regression in two out of five patients treated. On the whole, our results in patients selected for the presence of vERs are more satisfactory. These results suggest that attempting to block estrogen-mediated gene activation at the postreceptor level through administration of a progestin is reasonable. It is possible that increasing the dosage (megestrol can be used without major side effects at a dosage of up to 800 mg/day) or the use of more potent progestins could lead to further improvement of outcome.

On the whole, the favorable outcome of antihormonal treatment, particularly treatment with tamoxifen, is strongly dependent on the type of ER present in the hepatic tumor. It should be considered, however, that HCC superimposed on cirrhosis is the final step of a process involving the entire liver, a process that is typically multifocal and often associated with histologically demonstrable dysplastic lesions. These dysplastic lesions are already characterized by the same modification of ERs demonstrated in HCC (20). It could therefore be suggested that antiestrogen therapy oriented by receptor status could be tested in an earlier step of cirrhotic process, when dysplastic lesions become evident.

Table 1 *Doubling time of the volume of neoplastic lesions in patients with HCC, divided according to the type of liver ER transcripts*

Doubling time	HCCs with wtERs	HCCs with vERs
Without therapy, days (mean ± SD)	175 ± 94[a,b]	42 ± 11[a]
With therapy, days (mean ± SD)	401 ± 144[b]	344 ± 341

[a] $P < 0.0005$.
[b] $P < 0.005$.

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Erica Villa, Aisha Dugani, Emanuela Fantoni, et al.

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