Eur J Cancer Clin Oncol, Vol. 23, No. 11, pp. 1659–1664, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00

Response to Cyproterone Acetate Treatment in Primary Hepatocellular Carcinoma is Related to Fall in Free 5α-Dihydrotestosterone

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Abstract—*The male preponderance in cirrhotic patients with primary hepatocellular carcinoma (HCC) and the presence of androgen receptors in tumour tissue suggest possible benefit from anti-androgenic therapy. Twenty-five cirrhotic patients with irresectable HCC (23 male) were treated with cyproterone acetate (CPA) 300 mg daily. Hepatic ultrasound, alpha-fetoprotein and total and free sex steroid levels were monitored. Five patients had an objective response to therapy with a median duration of 8 weeks and survival in excess of 29 weeks. Median survival for all patients was 14 weeks. Apart from transient paranoia in two cases, side-effects were minimal. Total androgen levels (measured in 13 patients) had fallen significantly at 10 weeks, but free 5α-dihydrotestosterone (DHT) which had fallen by 4.8 pM (median) in five responders, had risen by 5.05 pM in eight non-responders: $P < 0.025$. The apparent correlation of response with reduction in free DHT suggests that hormonal manipulation may be effective in HCC if free DHT is reliably reduced. This has been achieved in other conditions by the combination of CPA with low dose oestrogen or with LHRH agonists.*

INTRODUCTION

In Western centres less than 10% of patients with primary hepatocellular carcinoma (HCC) are treatable by hepatic resection (including orthotopic transplantation) and in fewer than 30% of the remainder is there a response to conventional chemotherapy, at the cost of variably severe side-effects [1–3]. Even in those who respond initially, subsequent relapses tend to be resistant to chemotherapy, and cardiotoxicity remains a problem with doxorubicin and, to a lesser extent, with mitozantrone [2, 4].

The striking male preponderance among cirrhotic patients with HCC [5, 6] has suggested hormonal involvement in this tumour. In support of such an hypothesis, firstly there is a consistent relative excess of total and free 5α-dihydrotestosterone (DHT) over testosterone, in serum and tissue of cirrhotic patients with HCC [7] and, secondly, androgen receptors are present at high concentration in HCC tissue [8–11] where they appear functional [12]. DHT is the most potent natural androgen and is responsible for androgen receptor-mediated responses in man. Free levels of this steroid are mainly determined by the level of sex hormone binding globulin (SHBG) which binds sex-steroids in circulation [13].

The use of antiandrogens in the treatment of HCC appears a logical approach. Toxicity using such drugs in the treatment of prostatic carcinoma has been minimal and mainly attributable to antiandrogenic effects [14]. A therapeutic trial of cyproterone acetate (CPA)—a potent antiandrogen with progestogenic activity—in patients with HCC and underlying cirrhosis is reported in which the correlation between tumour response and the antiandrogenic effects of the drug has also been examined.

METHODS

Twenty-five patients (23 male) of mean age 58.7 years (median 63, range 33–73) with irresectable HCC complicating cirrhosis were studied (Table 1). In 19 cases the diagnosis of malignancy was confirmed histologically, and in the remainder

Accepted 1 July 1987.
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Table 1. Patient characteristics

Case	Sex	Age	Aetiology of cirrhosis	Histological proof of diagnosis	Hepatic decompensation	Okuda grade	Spironolactone therapy	Metastases
1	M	64	Alcohol	+	+	II	+	—
2	M	43	HBV	+	−	II	−	Pleura
3	M	56	Alcohol	+	+	II	+	—
4	M	43	Alcohol	+	+	II	+	—
5	M	44	HBV	−	−	II	−	—
6	M	52	Alcohol	+	+	III	+	—
7	M	65	Alcohol	−	−	II	−	—
8	M	63	HBV	+	−	I	+	—
9	M	51	Alcohol	+	−	II	+	—
10	M	66	Alcohol	+	−	I	−	—
11	M	55	Cryptogenic	+	−	I	−	—
12	F	66	PBC	+	−	II	−	—
13	M	42	HBV	−	−	II	+	—
14	M	70	HBV	+	−	III	−	—
15	M	70	Auto-immune	−	−	II	−	—
16	M	60	HBV	−	+	II	+	—
17	M	73	PHC	+	−	II	−	—
18	M	60	Alcohol	+	+	III	+	Peritoneum
19	M	64	Alcohol	+	+	II	+	—
20	M	65	Cryptogenic	+	−	II	−	—
21	M	72	HBV	+	−	II	−	Diaphragm
22	M	33	HBV	+	−	I	−	Lungs
23	F	65	PBC	+	+	II	+	—
24	M	62	Alcohol	−	+	III	+	—
25	M	64	Cryptogenic	+	−	II	−	Nodes

HBV: chronic hepatitis B virus infection; PBC: primary biliary cirrhosis; PHC: primary haemochromatosis.

tumour demonstrated on ultrasound scanning was shown to be HCC both by hepatic arteriography and by elevation of serum alpha-fetoprotein (αFP) (radioimmunoassay: Amersham International) to greater than 250 μg/l. Cirrhosis was related to chronic hepatitis B virus infection in eight patients (2: eAg + sAg, 5: eAb + sAg, 1: sAb), to alcohol in 10, to autoimmune chronic active hepatitis in one, to haemochromatosis in one, was cryptogenic in three and was from primary biliary cirrhosis in the two women (Table 1).

Presentation in 24 was with abdominal discomfort, weight loss and hepatomegaly, which was in association with features of hepatic decompensation in nine (Table 1). The remaining patient (Case 8) had presented with cardiovascular collapse secondary to spontaneous rupture of tumour requiring emergency surgery to achieve haemostasis. Twenty-three patients had received no previous therapy directed against the tumour, two had disease unresponsive to mitozantrone (cases 11 and 18), and 12 were on spironolactone for control of ascites (Table 1). One patient had previously been treated with penicillamine (Case 23) and another with prednisolone and azathioprine (Case 15). There was no other relevant medication history. Extra-hepatic metastases were detected in five patients (Table 1). In the system of grading for HCC suggested by Okuda [15] based on tumour size, the presence or absence of ascites, albumin and bilirubin levels, four patients were in Group I, 17 in Group II and in 4 in Group III (Table 1). WHO performance grade was recorded prior to and during therapy [16]. Patients were selected for the trial only by exclusion of those suitable for surgical management and of those expected to die within 48 h of diagnosis. Cyproterone acetate was initially administered orally at a dose of 100 mg thrice daily but this was later modified to phased introduction with 100 mg daily for 1 week, 100 mg twice daily for 1 week and 100 mg thrice daily thereafter. Full blood counts, serum biochemistry and liver size (cm below costal margin in mid clavicular line) were monitored weekly for 4 weeks and then monthly. Serum αFP was measured and ultrasound examination of the liver performed, at least 4-weekly, and tumour volume estimated from three perpendicular diameters as measured on ultrasound.

Toxicity was expressed according to the recommended WHO scales [17]. Response to therapy was assessed by serial measurements of estimated tumour volume, αFP level, WHO performance grade and regular assessment for the development of new metastases. Complete response was defined as return of performance grade and liver size to normal, disappearance of tumour on ultrasound

Table 2. Tumour volume, αFP and durations of response and survival in responding patients

Case	Pecentage fall in tumour volume	Percentage fall in αFP volume	Duration of response (weeks)	Duration of survival (weeks)
7	52	17	7	14
8	0	72	8	53
9	87	−0.5	12	>29
10	56	52	8	16.5
11	50	83*	26	32

*To within normal range.

and reduction of αFP level by 95%, all to last at least 4 weeks. Partial response required either 50% or more reduction in estimated tumour volume or 50% or more reduction in αFP level, lasting at least 4 weeks. Stable disease was defined as reduction of tumour volume or αFP not qualifying for partial response, or an increase in either of less than 25%, while progressive disease was diagnosed if volume or αFP rose by more than 25%, if new metastases appeared or with the patient's death.

In 13 patients (cases 7–12, 18, 19 and 21–25) circulating levels of testosterone, DHT, oestradiol and SHBG were measured prior to therapy and again after 1 month on full doses of CPA. These measurements were performed at 10 weeks except in case 18 who was commenced on 300 mg daily and where measurements were at 8 weeks. Serial assays were not possible in the remaining 12 cases because of death before a month on *full* dosage (cases 1–6, 13–17) and loss of sample from vial breakage (case 20). Radioimmunoassays for the three steroids were by the methods of Collins *et al.* [18], Buratti de Hoghton and Iqbal [19] as modified by Iqbal *et al.* [20] and Emment *et al.* [21] respectively, and SHBG levels were determined by the two-tier column ligand binding assay [22]. Levels of steroids free from SHBG and albumin were estimated by the method of Iqbal *et al.* [23]. Radiolabelled steroids were from Amersham International; unlabelled steroids and steroid antibodies were from Sigma Chemical Co.

Statistical analysis was with the Wilcoxon matched pairs signed ranks test or the rank sum test, as appropriate.

The trial had approval from the ethical committee of King's College Hospital and all patients gave informed consent. No eligible patient declined entry to the study.

RESULTS

Six patients died before the completion of 4 weeks therapy and were therefore unassessable (Cases 1–6) but are included for analysis of survival. Amongst the remaining 19 patients there were no complete responders but a partial response was seen in five (Table 2) and in a sixth there was a possible delayed response. In two patients response was confirmed by falls in both tumour volume and αFP, in two there was significant reduction in tumour size and αFP did not rise further, and in one the αFP fell with tumour size unaltered. The median (and mean) duration of response was 8 (12.2) weeks and duration of survival >29 (>28.9) weeks. (If linear rather than volumetric measurements of tumour size had been made then in only Case 7 would confirmation of response not have been possible.) In case 12 αFP and tumour size rose for 8 weeks but at 12 weeks the tumour volume was stable and the αFP level had fallen to 43% of the level at 8 weeks. This possible delayed response lasted a documented 6 weeks and was only terminated 2 weeks later by variceal haemorrhage complicated by hepatic failure which proved fatal. Progressive disease, as judged by clinical assessment, tumour volume and serum αFP, led to death within 2 months in six patients (Cases 13–18) but each of the remaining seven patients had a period of documented stable disease whilst on therapy. Overall median survival was 14 weeks (mean 14.4 ± 12.97) and that for evaluable patients was 19 weeks (18.1 ± 12.69).

Toxicity from therapy was a problem in only two patients (Cases 2 and 19) who developed acute paranoia after 14 and 3 days, respectively. Both had received 300 mg CPA daily from the onset of therapy, and in both cases all psychiatric symptoms resolved within 48 h cessation of therapy. Reintroduction at a dose of 100 mg daily was well tolerated in Case 19 but was not attempted in Case 2 in view of rapidly progressive disease. Tiredness and mild lethargy (WHO Grade 1) seen in eight patients, was ameliorated in three cases by taking the 300 mg as a single night-time dose—suggesting that it was drug related—and was not sufficient to require dosage reduction in the remainder. Diarrhoea (WHO grade 2), previously undescribed with CPA and probably unrelated to therapy, occurred in one patient: no dose reduction was necessary and symptoms resolved spontaneously after 3 days. Renal function and haematological parameters were unaffected and no other side-effects were observed.

In untreated patients, levels of SHBG were elev-

Table 3. Sex steroid profile before and on treatment with cyproterone acetate (median values; n = 13)

	Before treatment	On treatment	*P* value
Total testosterone (nM)	5.2	1.9	<0.025
Range	1.7–16.6	1.4–2.6	
Total DHT (nM)	1.4	0.75	<0.005
Range	0.72–3.2	0.55–1.0	
Total oestradiol (pM)	481	141	<0.005
Range	15–1073	15–719	
SHBG (nM)	52.8	31.1	<0.005
Range	10–90	8–57	
Free testosterone (pM)	137	122	NS
Range	12–332	63–178	
Free DHT (pM)	34	18.3	NS
Range	1.8–38	7.0–30	
Free oestradiol (pM)	62.3	19.2	<0.025
Range	2–161	2–94	

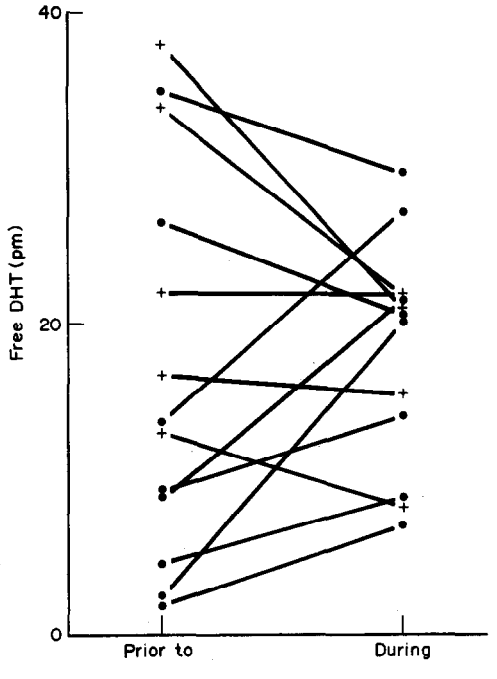

Fig. 1. Levels of free dihydrotestosterone before and whilst on full doses of cyproterone acetate: + — + responders; ●—● non-responders.

ated, and in the males total and free testosterone were low, and total and free oestradiol high (Table 3). The total DHT levels were normal but the free levels of this androgen also were low (Table 3).

At the 10 week 'landmark' [24] on therapy with CPA, levels of SHBG, total testosterone, total DHT and total and free oestradiol had fallen ($P < 0.005$, $P < 0.025$, $P < 0.005$ and $P = 0.025$, respectively) (Table 3). There was a striking dichotomy—significant at $P < 0.025$—in levels of free DHT between those who had shown a clinical response to CPA and those who had not (Fig. 1), falling in four of the five responders and unchanged in the fifth but rising in all but two of the non-responders. Neither the response to therapy nor the effects on total and free hormone levels appeared influenced by spironolactone given before and during the study (2/4 responders with a falling free DHT; 3/6 of those with a rising free DHT).

Study of outcome following the 10 week landmark in the 13 patients in whom steroid assays were done supports the relevance of response to survival, with a median of >19 further weeks in those showing a response at 10 weeks compared to 9.5 weeks in those not (NS). A falling free DHT at 10 weeks was a better discriminator of prognosis than response, with a median further survival of 20 weeks, compared to 9.5 weeks in patients where the free DHT had risen ($P < 0.05$).

DISCUSSION

The modest objective response rate observed with CPA (20% for all patients entered) is comparable to that expected with conventional cytotoxic chemotherapeutic agents [3] as is the median duration of survival seen here, despite the inclusion of patients in whom prognosis was expected to be poor and who would have been excluded from many of the reported series; the confidence limits for this response rate nevertheless remain wide.

The difference in sex-steroid profile whilst on therapy between those who did and did not respond is notable and appeared independent of sex and other prognostic indicators. The hormonal study only of patients surviving to at least 1 month on full doses of CPA is likely to have introduced a negative bias, diminishing the differences observed (Type II error). This difficulty is to some extent circumvented

by the separate analysis of post-landmark prognosis as advocated by Anderson *et al.* [24], which yields the same conclusions and suggests that falling free DHT is actually a better guide to prognosis than measurement of αFP and tumour volume. Although a potent antiandrogen and progestin, CPA also reduces levels of SHBG and it has been recognised previously [25] that it may thereby cause an increase or decrease in free androgens (as here) despite consistent reduction of total levels. A fall in free androgens may however be readily ensured (as for example in the management of hirsutism), by the addition to CPA of a small dose of oestrogen [25] or the use of an LHRH agonist [26] to maintain SHBG levels. The undoubted interest in examining serial samples of tumour tissue for androgen receptor levels during such a study is unfortunately unlikely to be satisfied in view of the ethical considerations.

Acute paranoia has not previously been recorded with CPA (Committee on Safety of Medicines Adverse Reaction Information Service) and, despite the lack of any correlation with the presence or absence of hepatic encephalopathy, may be related to underlying chronic parenchymal liver disease: it appears to be avoided by gradual introduction of the drug. Toxicity was otherwise minimal. The positive correlation of response with a fall in free DHT supports the view that HCC is an androgen responsive tumour and that efficacy of CPA is primarily related to its antiandrogenic activity rather than to its progestogenic effects.

The apparently successful treatment of HCC by medroxyprogesterone/megestrol acetate—three patients with documented partial remission [27]—and by tamoxifen when in combination with norethisterone—three patients with falling αFP albeit with stable tumour size [28]—but not when used alone [29], indicate that the progestogenic activity of CPA may also be beneficial.

A combination of CPA with low dose oestrogen or the use of an LHRH agonist to ensure a fall in the available/free androgen level offers an alternative approach for the treatment of HCC: the controlled comparison of such anti-androgenic therapy with conventional chemotherapeutic agents now appears justified.

Acknowledgement—We thank Schering Chemical (U.K.) Ltd. for financial support.

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