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Serial serum AFP heterogeneity changes in patients with hepatocellular carcinoma during chemotherapy

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Summary. Serum alpha fetoprotein (AFP) is heterogeneous, one form binding to the lectin concanavalin A (conA) and the other not. The relative amounts, of the two forms in the serum of patients has diagnostic applications in differentiating between primary hepato-cellular carcinoma and metastatic liver disease. In 36 patients with primary hepatocellular carcinoma, the conA-nonreactive form of AFP comprised less than 20% of the total (range 1.6%–19.2%; median 8.7%), whereas in 13 patients with metastatic liver disease the conA-nonreactive form comprised more than 20% of the total (range 26.6%–91.7%; median 57.6%).

Four patients with primary hepatocellular carcinoma were treated with CB3717, and serial changes in the serum AFP characteristics were examined. In two patients in whom the total serum AFP concentration fell, the percentage of the conA-nonreactive fraction, initially less than 20% rose steadily. In two other patients the total serum AFP did not fall significantly and the proportion of the conA-nonreactive fraction remained below 20%.

Introduction

Affinity chromatography of serum alpha fetoprotein (AFP) from humans and rats on concanavalin A (conA) Sepharose yields two types of isoprotein, a conA-reactive fraction which binds to the lectin and a conA-nonreactive fraction which does not [3, 4, 8, 9]. The reactive and nonreactive fractions are thought to originate from the fetal liver and the yolk sac, respectively [5, 7, 8, 10]. Using this differential reactivity, we have demonstrated a rational means of distinguishing between primary hepatocellular carcinoma and hepatic secondaries [3]. The nonreactive AFP fraction comprised less than 20% of the total serum AFP in patients with histologically proven primary hepatocellular carcinoma, but was greater than 20% in patients with histologically proven hepatic secondaries.

In the present communication the series has been extended with a further 15 cases of primary hepatocellular carcinoma and 5 cases of metastatic liver disease. We have also investigated the changes that occur in serum AFP heterogeneity in patients with primary hepatocellular carcinoma during chemotherapy with the thymidylate synthetase inhibitor CB3717.

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Patients and method

Sera were obtained from 15 patients with primary hepatocellular carcinoma and 5 patients with metastatic liver disease. The samples were stored at −20 °C until analysed. The diagnoses were established by histological examination of biopsy material. Four patients with primary hepatocellular carcinoma were followed with serial estimations during the course of chemotherapy. Affinity chromatography of AFP was carried out as previously described [3]. Portions (0.2 ml) of each serum sample were applied to columns with 5.0 ml bed volume of conA-Sepharose AB, which had been previously equilibrated with Tris-HCL (50 mmol/l, pH 7.5) containing 1 mmol/l NaCl, 1.0 mmol/l CaC12, 1.0 mmol/l $MgCl_2$, and 0.2 ml. Tween 20 wetting agent, and eluted with the same buffer. The initial 3.0 ml eluate was discarded, and the subsequent 5.0-ml fraction containing the conA-nonreactive AFP was collected for analysis. The columns were regenerated with 15 ml equilibrating buffer containing 0.1 mmol/l glucose.

The percentage of AFP that was nonreactive was estimated from the formula:

$$\frac{\text{AFP } \mu\text{g/l column eluate} \times 5}{\text{AFP } \mu\text{g/l serum} \times 0.2} \times 100 \ .$$

The total and nonreactive AFP concentrations were estimated with the aid of AFP-EIA kits from Abbott Laboratories Limited, Diagnostic Division, The Business Centre, Molly Millars Lane, Wokingham, Berkshire, RG11 2QZ. The assay has a dynamic range of 5–400 g/l and a coefficient of variation of less than 8%.

Treatment. The patients were treated with CB3717, a potent quinazoline antifolate inhibitor of thymidylate synthetase [6]. The drug was given IV (300 mg/m^2) at 3-weekly intervals. One patient's chemotherapy was changed after two courses of CB3717 to etoposide (VP16) and adriamycin, because of poor response to CB3717. Etoposide, which inhibits the incorporation of thymidine into DNA or destroys cells in the G2 phase [1], was given at a dose of 150 mg daily for 3 days. Adriamycin (60–75 mg/m^2) was administered at 3-weekly intervals as an IV bolus during a 5% dextrose infusion (500 ml) given over a period of 1–2 h. Perchlorperazine maleate (25 mg) was administered IV before each dose of adriamycin.

Table 1. Summary of percentage conA-nonreactive AFP in patients with primary hepatocellular carcinoma and metastatic liver disease

	Percentage conA-nonreactive AFP[a]			Percentage conA-nonreative AFP[b]		
	No. of cases	Median	Range	No. of cases	Median	Range
Primary hepatocellular carcinoma	15	10.2	2.8–19.2	36	8.7	1.6–19.2
Metastatic liver disease	5	57.7	51.8–91.6	13	57.6	26.6–91.7

[a] Recent data
[b] Total number of cases studied

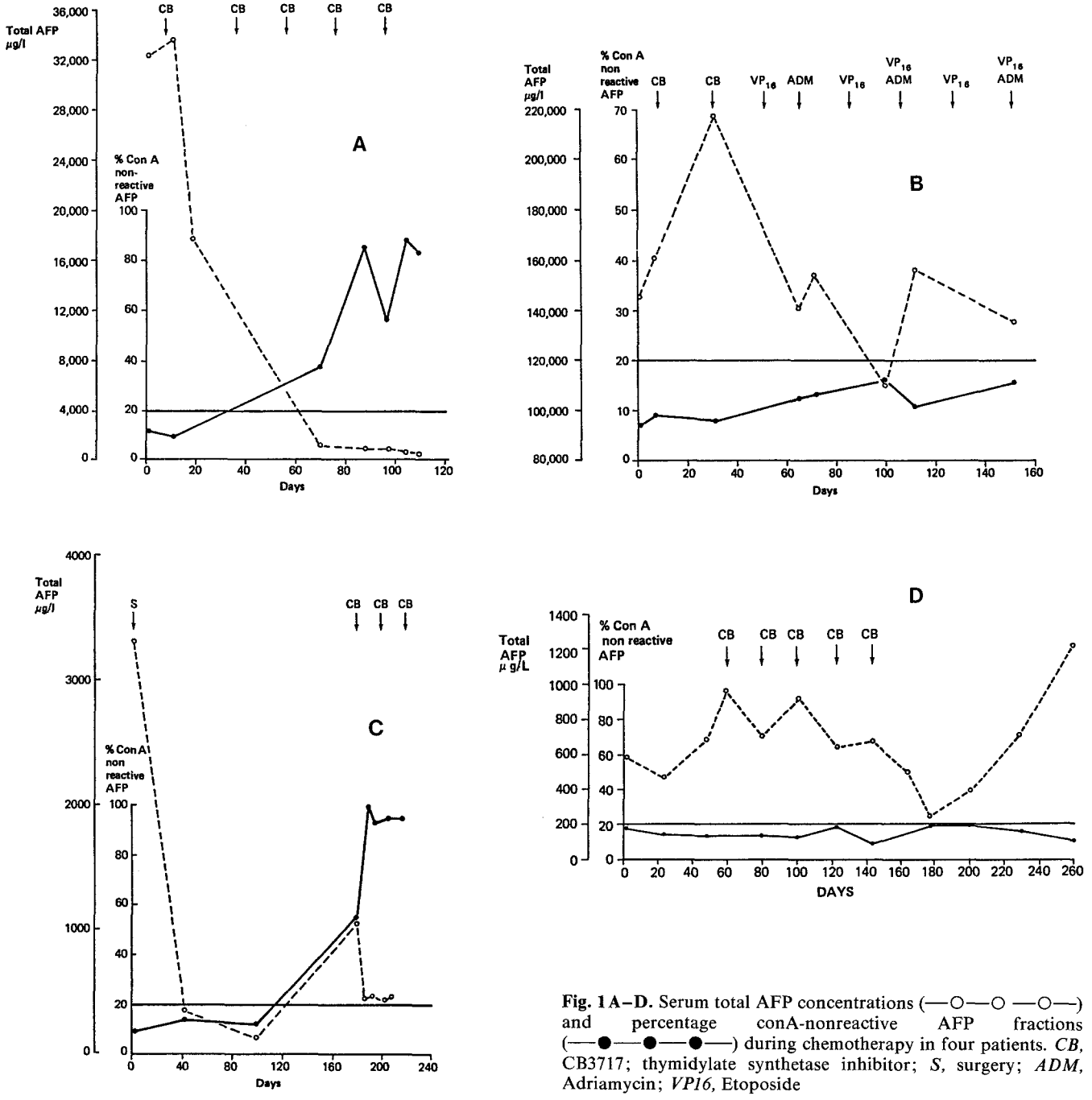

Fig. 1A–D. Serum total AFP concentrations (—○—○ —○—) and percentage conA-nonreactive AFP fractions (—●—●—●—) during chemotherapy in four patients. *CB*, CB3717; thymidylate synthetase inhibitor; *S*, surgery; *ADM*, Adriamycin; *VP16*, Etoposide

Results and discussion

The results of recently studied patients (15 with primary hepatocellular carcinoma and 5 with metastatic liver disease) are summarised in Table 1A. The conA-nonreactive fraction of AFP in serum from patients with primary hepatocellular carcinoma ranged from 2.8% to 19.2% of the total, with a median value of 10.2%. In metastatic liver disease the values ranged from 51.8% to 91.6%, with a median value of 57.7%. The results of all cases analysed to date are summarised in Table 1B. The recent data confirm the original assertion that serum AFP heterogeneity is a useful means of differentiating between patients with AFP-producing primary hepatocellular carcinoma and metastatic liver disease.

For four patients receiving chemotherapy, the total serum AFP concentrations and the conA-nonreactive percentages are presented graphically in Fig. 1. With two patients (A and C) the total AFP concentrations showed a dramatic fall after starting chemotherapy with CB3717. This in itself indicated the efficacy of the treatment, and the marker level correlated well with clinical assessment. Examination of the relative proportions of the serum AFP fractions in serum shows that the conA-reactive form is more sensitive to chemotherapy. In both patients (A and C) the percentage of the AFP that was conA-nonreactive was less than 20% of that at the beginning of chemotherapy. As the total AFP concentration fell, the conA-nonreactive AFP fraction rose and remained above 20%. This might represent a change in the nature of the tumour cells. It is also clear that with patients B and D chemotherapy was largely ineffective, as the total serum AFP concentration was not significantly different from that before treatment. The concentration of conA-nonreactive AFP also showed little change. We have previously shown that human serum AFP has two isoproteins, a conA-nonreactive AFP fraction, which is abundant in the sera of patients with germ cell tumours [4], and another form, which binds to conA and is prevalent in the sera of patients with primary hepatocellular carcinoma [3]. Our results provide evidence that both isoproteins of AFP are produced by patients with primary hepatocellular carcinoma.

It has been found that changes in the glycosylation of a number of individual cell surface glycoproteins accompany malignant transformation [2, 11]. It is possible that the differences we have demonstrated in the serum AFP in these patients with primary hepatocellular carcinoma undergoing chemotherapy reflected a general phenomenon of alterations that take place in the glycosylation of a given protein, depending on the stage of differentiation of the cell or tumour producing it. This enables us to speculate that during chemotherapy the tumour cells undergo differentiation or a new clone of cells emerges.

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