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A Phase I/II Trial of Epirubicin and High Dose Tamoxifen as a Potential Modulator of Multidrug Resistance in Advanced Hepatocellular Carcinoma

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HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignancies, causing more than 1 million deaths every year, worldwide [1]. Because most patients present with advanced disease and HCC is almost always associated with chronic underlying liver disease, potential curative surgery can be achieved in only a small percentage of cases. There are numerous palliative treatment options in patients with inoperable disease, although none have yet been shown to offer any reproducible therapeutic benefit [2]. The possible explanations for the refractoriness of HCC to chemotherapy include tumour heterogeneity, inherent resistance and the overexpression of the multidrug resistance (*MDR1*) gene [3]. Based on histological studies, indicating that HCC expresses high levels of P-glycoprotein (Pgp) [3, 4], and the resistance-reversing potential and tolerance of high dose tamoxifen [5, 6], which has also been reported to exert therapeutic activity as a hormonal agent in this disease [7], the present disease-oriented phase I study was initiated.

For inclusion in the trial, patients were required to be aged 70 years or younger, have a WHO performance status of < 3, and adequate renal (serum creatinine level < 1.5 mg/dl), liver (total bilirubin level < 2 mg/dl, transaminase levels less than twice the upper limits of normal) and bone marrow functions (leucocyte count > 4000/μl, platelet count > 100 000/μl). All patients had normal pretreatment electro- and echocardiograms (with a left ventricular ejection fraction of more than 50%), and provided informed consent according to institutional guidelines.

Treatment consisted of tamoxifen 80 mg given twice a day for a total of 9 days, and the cytotoxic agent epirubicin, which was administered as a continuous infusion over 24 h

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Table 1. Haematological toxicity

Epirubicin dose level (mg/m^2)	Number of patients/ evaluable cycles	WHO grade 4 (median nadir counts $\times 10^9/l$) Leucopenia	Granulocytopenia	Thrombocytopenia
1st two cycles (in determination of the MTD)				
70	3/6	0 (5.90)	0 (4.01)	0 (136)
80	3/6	0 (3.90)	0 (1.86)	0 (227)
90	3/6	0 (4.80)	0 (2.20)	0 (168)
100	6/12	0 (2.11)	3 (1.13)	0 (174)
110	6/10	2 (3.70)	4 (2.40)	0 (104)
All treatment cycles (including dose-reduced cycles)				
70	3/7	0 (5.90)	0 (4.85)	0 (155)
80	3/10	0 (4.20)	0 (2.36)	0 (198)
90	3/17	0 (3.84)	0 (2.09)	0 (167)
100	15/47	0 (2.90)	8 (1.70)	1 (176)
110	6/13	2 (3.10)	4 (1.80)	0 (114)

WHO, World Health Organization; MTD, maximum tolerated dose.

on day 8 of the cycle. Treatment was repeated every 4 weeks for a total of six cycles, unless there was objective evidence of disease progression. The starting dose of epirubicin was 70 mg/m^2, and dose levels were escalated in consecutive cohorts of 3–6 patients to 80, 90, 100 and 110 mg/m^2, utilising an escalating-dose phase I design. In order to determine the maximum tolerated dose (MTD), the first two treatment cycles were evaluated. MTD was defined as the dose level below that producing dose-limiting toxicity (DLT), i.e. any WHO grade 4 haematotoxicity (in at least 4 of 6 patients) or grade 3/4 toxicity except alopecia in a specific organ. No intrapatient dose escalation was allowed. In case of grade 4 haematological or any other severe organ toxicity in individual patients, the epirubicin dose was reduced one level for subsequent cycles. Measurable disease was reassessed every two cycles. Toxicity and therapeutic efficacy were evaluated according to WHO standard criteria.

Between November 1992 and March 1994, 30 patients were entered into this protocol, all of whom were considered evaluable for assessment of toxicity and therapeutic response. 7 patients were female and 23 male, with a median age of 66 years (range 40–70). The median WHO performance status was I. Cirrhosis was present in 25 (83%) patients, the large majority of whom had well-compensated liver function (22 and 8 patients had Child-Pugh class A and B, respectively). Disease was confined to the liver in 24 patients, 4 had pulmonary metastases, and 2 patients had bone-, one combined with soft tissue involvement. A total of 94 treatment courses (median 3; range 1–6) was administered to the patients at doses of 70–110 mg/m^2 epirubicin. The number of patients and courses per dose level, as well as the haematological toxicity observed during the first two courses in determination of the MTD and during all subsequent treatment cycles are shown in Table 1. Myelosuppression was the dose-limiting toxicity with an MTD of 100 mg/m^2 epirubicin. None of the patients at the first three dose levels experienced any severe toxicity, although 3/6 and 4/6 patients had grade 4 granulocytopenia during their first two treatment courses at levels 100 and 110/m^2, respectively. Apart from granulocytopenic infections, which were recorded in 5 of 30 patients and required hospitalisation in 2, the most prominent non-haematological adverse reactions were alopecia ($n = 30$) and local toxicity at the injection site in patients without a central venous access (4 of 12 patients who received chemotherapy via peripheral veins had grade 1–2 phlebitis and 2 required surgical necrosectomy due to drug extravasation). Cardiotoxicity was not detected by ECG-monitoring and serial echocardiograms, and we did not observe any high dose tamoxifen-related adverse reactions, except for 1 patient with reversible vertigo. None of the 21 patients, who were treated in the escalating-dose phase I stage of the study, and only 1 of 9 patients, who were subsequently treated at the MTD, achieved objective remission to this therapy. Stable disease was noted in a total of 14 (47%) patients, and the tumour was progressive in 15. The median time to progression in all 30 evaluable patients was 3.5 months (range 3–9.5), and median survival was 5.8 months (range 1–16).

Thus, while the combination therapy of high dose tamoxifen and protracted infusional epirubicin is feasible and can be safely administered to patients with unresectable HCC, we were unsuccessful in circumventing drug resistance.

There are several possible explanations for the disappointing therapeutic outcome, including a heterogeneous overexpression of P-glycoprotein within a given population of tumour cells, use of an inadequate dose of tamoxifen to assure effective competition for binding sites in patients with large tumour volumes, and most likely, more complex forms of drug resistance involving mechanisms other than Pgp may be operative [8].

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Serum and Urinary Vascular Endothelial Growth Factor Levels in Non-small Cell Lung Cancer Patients

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INDUCTION OF new capillary blood vessels is required for tumour growth [1]. At the beginning of their development tumours are not vascularised and the induction of an angiogenic phenotype switches tumours to a more aggressive behaviour. Angiogenesis is mediated by specific angiogenic peptides (basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), tumour necrosis factor-α (TNFα)) probably released by tumour cells and tumour-associated macrophages [2–4]. The angiogenic peptides are mitogenic for several tissues, with the exception of VEGF, which is specific for endothelial cells [5]. VEGF is also a potent vascular permeabilising agent which causes leakage of plasma proteins with formation of a provisional stroma that allows the migration of endothelial cells and fibroblasts. VEGF is a 34–45 kDa protein which exists in four different isoforms of 121, 165, 189 or 206 amino acids. It is expressed in many normal tissues and is overexpressed in many non-neoplastic diseases (psoriasis, rheumatoid arthritis and ischaemic heart disease) [6]. Together with its two receptors (flk-1 and KDR), VEGF is highly expressed both at the mRNA and protein level in tumour cells and in endothelial cells of new borne vessels [7].

In several studies, raised serum levels of angiogenic peptides are associated with poor prognosis [8]. In order to study the relationship between VEGF levels in biological fluids and non-small cell lung cancer (NSCLC), we measured VEGF levels in serum and urine of 40 patients (mean age ± S.D., 62.5 ± 8.5 years) with newly diagnosed NSCLC (stage I:10; stage II:8; stage IIIA:8; stage IIIB:7; stage IV:7) and in 18 healthy controls (mean age ± S.D., 40 ± 12 years).

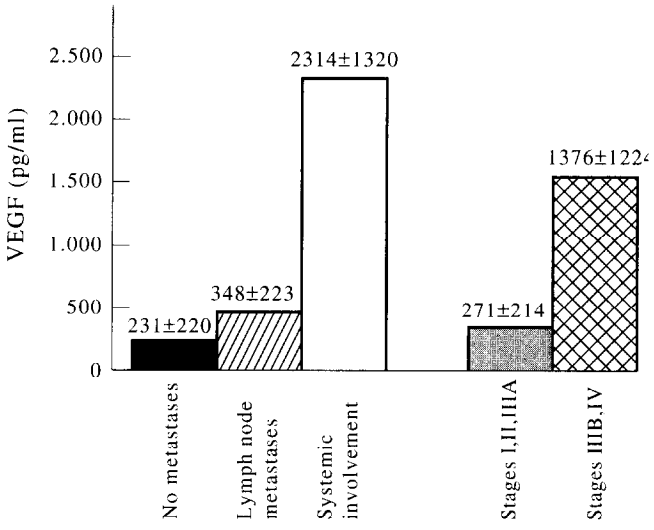

Figure 1. Relationship between serum VEGF and extent of disease.

Serum samples were collected by venipuncture, centrifuged and then stored at −70°C until assayed. VEGF levels in serum and in urine were determined using an immunoenzymatic assay where samples and biotinylated cytokine compete for the same antibody binding site. Measurements were made at a wavelength of 492 nm. Data are expressed as mean ± standard deviation (S.D.), and were statistically analysed using the Student's *t*-test. P values < 0.05 were considered statistically significant. The mean value of serum VEGF in healthy controls was 66.01 ± 96.22 pg/ml, with a significant difference between males (12.50 ± 6.53 pg/ml) and females (133.35 ± 114.7 pg/ml) ($P = 0.036$). In NSCLC patients, serum VEGF ranged from 38.96 to 4275 pg/ml and the mean value (602 ± 847.29 pg/ml) was significantly higher than mean VEGF values of healthy controls ($P = 0.01$). We considered 'raised' VEGF levels those ⩾ the mean + 3 S.D. of healthy controls (355 pg/ml); the percentage of patients with 'raised' VEGF increased from 39% for stage I to 100% in stage IV (50% in stage II, 62.5% in stage IIIA and 86% in stage IIIB). In the whole group, patients with systemic involvement (2315 ± 1320 pg/ml) showed a significantly higher mean serum level than patients without lymph node or systemic involvement (231.06 ± 220.73 pg/ml; $P < 0.001$). Although patients with lymph node involvement showed a higher mean value (348.01 ± 223.80 pg/ml) than patients without lymph node involvement, the difference was not statistically significant (231.06 ± 220.73 pg/ml, $P = 0.135$). Moreover, patients with advanced disease (stage IIIB and IV) (1376.08 ± 1224.84 pg/ml) had a higher mean value than that of early stage patients (271.03 ± 214.22 pg/ml) ($P < 0.001$) (Figure 1). Urinary level in healthy controls was 1.226 pg/ml and 2.26 pg/ml in NSCLC patients (P = n.s.); a statistical difference was found only between normal subjects and patients with stage IV disease (4.52 ± 2.59 pg/ml; $P = 0.010$).

In conclusion, VEGF serum levels appear to be increased in patients with NSCLC; these preliminary data show a re-

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