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Original Paper

Epirubicin and Etoposide Combination Chemotherapy to Treat Hepatocellular Carcinoma Patients: a Phase II Study

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Approximately half the patients affected with hepatocellular carcinoma (HCC) present with unresectable disease, so that efficacious systemic chemotherapy protocols are badly needed. We report the results of a phase-II study aimed at testing the efficacy and toxicity of a combination of epirubicin and VP-16.

Thirty six patients (30 men and 6 women) received epirubicin (40 mg/m^2, on day 1) and VP-16 (120 mg/m^2, on days 1, 3 and 5) every 28th day. Chemotherapy was stopped in case of disease progression, while the patients who achieved an objective response or who had stable disease continued treatment for a maximum of 10 cycles. One patient (3%) achieved a complete response, while 13 patients (36%) achieved partial response, i.e. 14 objective responses in all (39%, 95% CI: 23–55%). 11 patients (31%) exhibited stable disease, while in the other 11 patients (31%) the disease progressed. Median overall survival time was 10 months and 13.5 months in the subgroup of patients responding to treatment. Significant, especially haematological, toxicity was documented, but in no case was it so severe as to require discontinuation of treatment or reduction of the dosage. In conclusion, this combination appears to be an active and tolerable therapeutic option for HCC patients who are not candidates for surgical or locoregional procedures, and in our opinion it deserves further exploration within a randomised controlled trial versus best supportive therapy. © **1997 Published by Elsevier Science Ltd.**

Key words: hepatoma, chemotherapy, epirubicin, VP-16, unresectable disease, phase-II study
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INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC), a neoplasm once thought to be limited to Eastern countries, is now on the increase even in low- and medium-risk countries [1]; it has been estimated that, in 1996 in the U.S., there will be more than 19500 new cases of HCC, and 15200 deaths due to this cancer [2].

Systemic chemotherapy has always been considered a poor therapeutic approach to HCC, with the best results obtained with surgical resection [3, 4], percutaneous ethanol injection [5] and arterial chemoembolisation [6], especially for small HCCs [7]. However, according to the data from the ninth National Survey of Primary Liver Cancer in Japan, no more than 20% of HCC patients undergo surgical resection, the only possible cure for this condition, with nearly 50% of patients started on systemic chemotherapy [8]. These findings stress the pressing need of efficacious systemic chemotherapy protocols for inoperable HCC patients.

Several clinical studies investigating the antitumour activity of different antiblastic drugs in HCC patients have yielded mostly poor results: indeed, few drugs have exhibited antitumour activity and objective response rates have rarely exceeded 15% in Far East countries [9], the figures being lower in the Western World [10].

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We report the results of a phase-II study designed to test the antitumour effectiveness and toxicity of a combination of epirubicin, one of the more efficacious single agents tested in these patients [10], and etoposide (VP-16), a semi-synthetic derivative of the podophyllotoxin which, despite being used with interesting results in this type of cancer more than 15 years ago [11, 12], has since received little attention.

PATIENTS AND METHODS

Patients

Inclusion criteria consisted of: histologically confirmed diagnosis of HCC; the presence of measureable but inoperable lesions; 0,1 or 2 ECOG performance status [13]; at least 2 months life expectancy; age under or equal to 75 years; no previous treatment (chemotherapy, radiation therapy, immunotherapy or embolisation); adequate blood counts (leucocytes $>3.0 \times 10^3/\mu l$, platelets $>60 \times 10^3/\mu l$); normal renal function (creatinine <1.5 mg/dl); normal cardiac function; no ongoing infections; adequate bilirubin levels (<3 mg/dl); no portosystemic encephalopathy; and no bleeding or recently-bled oesophageal varices. Such inclusion criteria are in agreement with Liver Cancer Study Group of Japan guidelines [9].

Before accrual, all patients were submitted to the following investigations: complete serum biochemistry, including alpha1-fetoprotein titration, EKG, chest X-ray (2 projections), abdominal CT and echocardiography.

Each patient was staged with the staging system suggested by Okuda and associates in 1985 (Table 1) [14]. Two expert surgeons from our university independently pronounced each patient inoperable based on tumour mass extent (invasion of both lobes, neoplastic venous thrombisation) or on the association of severe cirrhosis with poor residual liver function.

Treatment

Epirubicin was administered at a dose of 40 mg/m^2, on the first day of each cycle, while VP-16 was administered at the dose of 120 mg/m^2, on days 1, 3 and 5 of each cycle. The cycles were repeated every 28th day.

All patients gave their informed consent to enrolment in this study according to institutional requirements and were treated on an outpatient day-hospital basis; after the first evaluation, at the end of the third cycle, chemotherapy was stopped in the case of disease progression, while the patients who achieved an objective response or who had a stable disease continued treatment for a maximum of 10 cycles.

Follow-up

Complete serum biochemistry and EKG were performed before each treatment cycle, while haematological and liver function tests were also evaluated on the 14th day of each cycle. Chest X-ray, echocardiographic evaluation of left ventricular function and abdominal CT to assess response were performed every 3 months.

Complete response (CR) required the disappearance of all perceptible tumour; partial response (PR) was defined as a 50% reduction in the product of the largest perpendicular diameters of the most clearly measurable known malignancies with no increase in the size of other measurable masses and no appearance of new lesions. Duration of response was calculated from the time the response began until progression. Stable disease (SD) required no change in size of the measurable lesions or a decrease in tumour size >50% or an increase <25% with no appearance of new lesions. Progression (P) was defined as the appearance of any new lesion and/or the growth of any existing lesion by ≥25% from the start of treatment.

Time to progression curve was plotted considering, for each patient, the time interval between treatment initiation and the first evidence of disease progression. Toxicity was evaluated according to commonly accepted WHO criteria [15].

Table 1. HCC staging by Okuda [14]

HCC/non-neoplastic liver		Ascitis		Albumin (g/dl)		Bilirubin (mg/dl)	
<50%	≥50%	NO	YES	≥3	<3	<3	≥3
–	+	–	+	–	+	–	+

Stage I: 0 (+); Stage II: 1 or 2 (+); Stage III: 3 or 4 (+).

RESULTS

Patients

The clinical features of the 36 patients enrolled in this study between November, 1993 and October 1995 are shown in Table 2. No cases of fibrolamellar (glassy cell type) hepatocellular carcinoma were diagnosed at biopsy, a type some authors consider as having better prognosis [16]. All the patients enrolled could be assessed for both response and toxicity.

Effectiveness

One patient (3%), after the 6th cycle, achieved a CR lasting 7 plus months, while 13 patients (36%) achieved PR, i.e. 14 objective responses in all (39%, 95% CI: 23–55%); mean duration of partial responses was 7.4 months (range: 6–12 months). Eleven patients (31%) exhibited SD, while in the other 11 patients (31%) the disease progressed, as demonstrated by enlarged and/or more measurable lesions.

For α_1-FP levels, the course of which usually reflects treatment efficacy, only 3 patients who responded to treatment presented with high titres, and treatment caused a

Table 2. Clinical features of the patients in our series

Men	30 (83%)
Women	6 (17%)
Mean age (years, range)	64 (51–75)
ECOG Performance Status (PS)	
0	6 (17%)
1	20 (56%)
2	10 (28%)
HBsAg + patients	8 (22%)
Anti-HCV + patients	13 (36%)
HBsAg and anti-HCV + patients	5 (14%)
Chronic alcohol abuse*	8 (22%)
Okuda stage	
I	12 (33%)
II	20 (56%)
III	4 (11%)
α_1 FP > 400 ng/ml	9 (25%)
α_1 FP < 400 ng/ml	27 (75%)

*Chronic alochol abuse: ≥80 g/day for ≥5 months.

Table 3. Response to treatment according to Okuda stage

	Stage I (n = 12)	Stage II (n = 20)	Stage III (n = 4)
Objective responses (CR + PR)	8 (67%)	5 (25%)	1 (25%)
Stable disease	3 (25%)	7 (35%)	1 (25%)
No response	1 (8%)	8 (40%)	2 (50%)

dramatic decrease in the level of the tumour marker in all of them.

The patient who achieved CR underwent a total of 10 chemotherapy cycles, while the patients judged as having PR and SD received an average of 9.6 and 7.6 cycles, respectively. Response according to Okuda Stage is shown in Table 3. One stage I patient, 8 stage II and 2 stage III progressed after 3 cycles despite treatment. Objective responses were observed in 3 patients presenting with >50% liver involvement and 6 cases who presented with a tumour mass involving <50% but > 25% of liver parenchyma, which, in our opinion, stresses the effectiveness of our treatment even in large tumours.

Of the responding patients, 5 patients were judged as having a PS of 0, 6 as PS 1 and 3 as PS 2.

Median survival time was 10 months (mean: 10.08 ± 3.96 S.D., range: 3–22) and it reached 13.5 months in the subgroup of patients responding to treatment (mean: 13.78 ± 2.94 S.D., range: 10–22); average time to progression was 7.25 months, but it reached 9 months when only the responding patients were taken into account. Both overall survival and time to progression curves are plotted in Figure 1. The total number of chemotherapy cycles we administered was 252, with a mean of 7 cycles a patient (range: 3–10 cycles).

Toxicity

The treatment caused significant haematological toxicity, especially in the patients receiving the highest cumulative doses of therapy. Indeed, of the 25 patients who received 6 or more chemotherapy cycles, 15 experienced grade III leucopenia on day 14 during 1 or more cycles; in all these patients oral prophylactic antibiotics were administered and further haemochromes were performed before the next scheduled treatment. Three of them required haemopoietic growth factor support to continue treatment, while no cases of febrile neutropenia were observed. The remaining 11 patients experienced at least one episode of grade II leucopenia.

Of the 11 patients who progressed after 3 cycles and therefore stopped treatment, only one experienced grade III, and 5 grade II leucopenia on day 14 of one or more cycles. Only one patient had grade III thrombocytopenia, while 16 other patients had grade II thrombocytopenia one or more times. However, it has to be stressed that 19 of our 36 patients were enrolled into this study with a

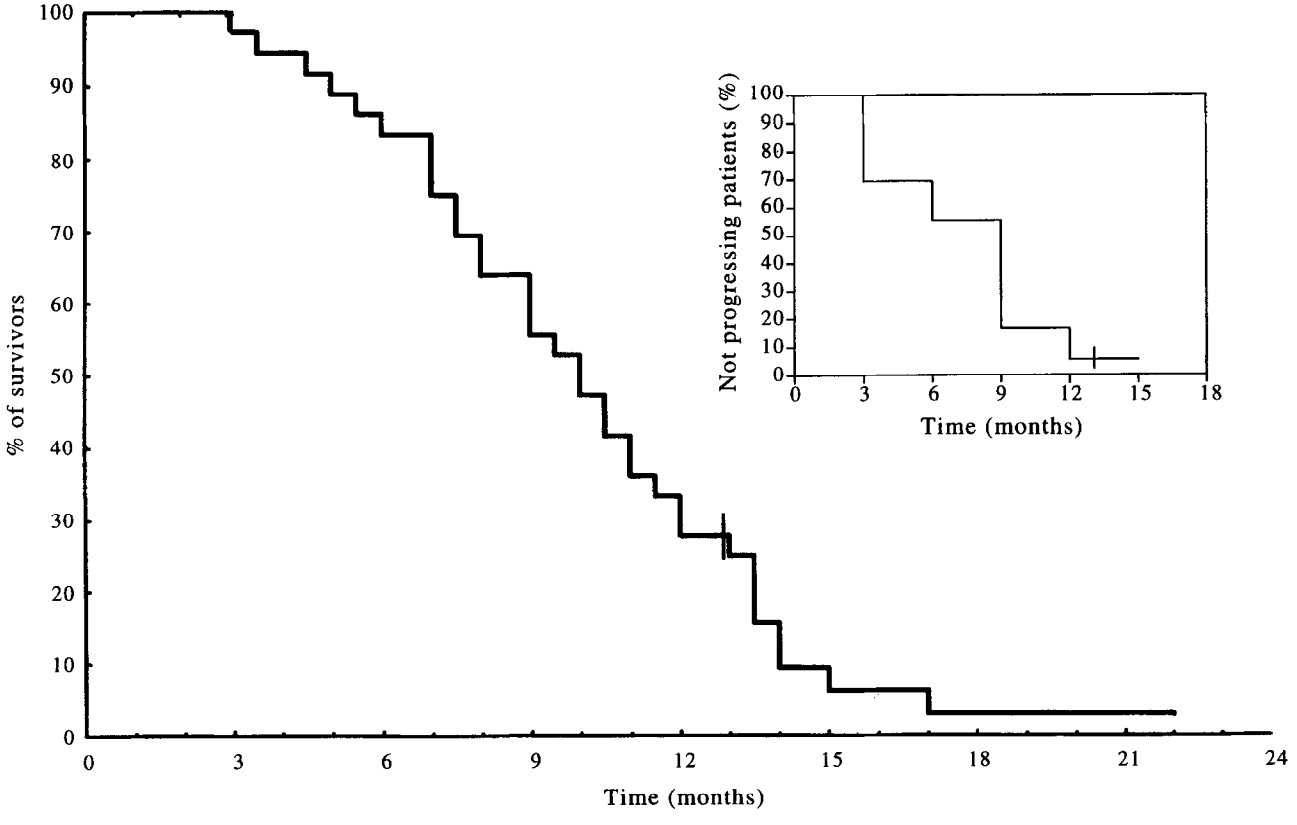

Figure 1. Kaplan–Meier curve of overall survival. The inset shows the time to progression curve at 3-monthly intervals, corresponding to the scheduled interval for follow-up for patients not progressing.

platelet count of less than $130 \times 10^3/\mu l$ due to pre-existing cirrhosis.

All patients complained of alopecia, 19 experienced grade II nausea during treatment, 8 had grade II mucositis and 1 patient had a reduction of left ventricular fraction after 9 cycles and a cumulative dose of 360 mg/m^2 of epirubicin, leading to treatment discontinuation. In no other case was treatment discontinued or the dosage reduced due to toxicity.

No toxic deaths due to acute liver failure or to other causes were recorded during treatment.

DISCUSSION

Systemic chemotherapy is still the main modality available for palliation for most HCC patients with inoperable tumours. A number of single agents and drug combinations have been given to patients in an attempt to alter their predictably short survival time, usually with poor results.

Despite often being used at a suboptimal dose, schedule or route [18], 5-fluorouracil (5-FU), the cornerstone of the treatment of enteric malignancies, has been broadly administered to these patients in the past, mainly with discouraging results, as clearly indicated by a resulting survival time hardly ever exceeding 2 months [10]. A significant antitumour activity of 5-FU modulated by leucovorin has been demonstrated only recently [19].

Currently, the most consistently effective agents against HCC are anthracyclines; in a comprehensive review by Nerenstone and associates a median response rate of 17% was documented in 644 patients in 13 published studies with these agents [10], ranging from a 80% response rate originally reported by Olweny [20], to no responders in another study [21]. However, once again, survival appeared not to be influenced by treatment, as demonstrated by the poor resulting survival of these patients, usually ranging from 2 to 5 months [10], with the sole exception of the Olweny study which reported 8 months survival [20].

VP-16 is another agent which has shown significant antitumour activity against HCC both *in vitro* and *in vivo* [11, 12]; in a dated comparative study of VP-16 and doxorubicin, the two drugs showed similar response rates [12]. Moreover, P-glycoprotein, an energy-dependent drug-efflux pump that reduces intracellular drug accumulation, thereby causing resistance to many structurally different chemotherapy agents, seems to be less effective in reducing VP-16 activity, at least *in vitro* in hepatoma cell lines [22]. HCCs are known to have elevated levels of *MDR1* gene expression [23], encoding for P-glycoprotein, and their drug resistance may be related to *MDR1* gene-mediated multridrug resistance. Therefore, VP-16 seems to be an appealing drug to test in such neoplasms.

The combination of an anthracycline and VP-16 to treat HCC patients was advocated by Melia and associates in 1983 [12]; although, to our knoweldge, the combination has been rarely used. In 1986, Giaccone and associates reported a complete response yielding a long-term survival (more than 2 years) in a single HCC patient treated with a combination of doxorubicin and etoposide [24].

The present study's results appear to be extremely interesting: a 39% objective response rate is a higher figure than those reported in several trials of different systemic chemotherapy protocols for HCC, even when anthracyclines have been included [9, 10, 18]. Moreover, even though our patients presented mainly with a favourable Okuda stage and with a good performance status, the overall median survival time we obtained (10 months for all patients and 13.5 for responders) appears extremely encouraging, being higher than the median survival time of both 229 untreated (8.3 months) and 55 chemotherapy-treated (4.3 months) Okuda I patients originally reported by Okuda and associates in 1985 [14]. Indeed, lower overall survival rates for HCC patients not candidates for surgical resection or for percutaneous alcohol injection have been reported in the literature [25].

The relevant patient selection in our study, with patients mainly in a favourable Okuda stage, is a direct consequence of the exclusion of any patient with bilirubin exceeding 3 mg/dl. Indeed, it is broadly known that an elevated serum bilirubin level is an adverse feature, which increases the toxicity of anthracyclines [26] and decresses the likelihood of tumour response to chemotherapy [26, 27]. The frequent reduction in anthracycline dosage due to high serum bilirubin levels, based on the view that the risk of myelosuppression is thereby lessened, may account for such decreased efficacy [27]. Benjamin and associates [26] suggested that the dose of anthracycline (in that case doxorubicin, given at the dose of 60 mg/m^2 every 3 weeks) be reduced by 50% for bilirubin levels between 1.2 and 3 mg/dl; in contrast, we decided to give the full epirubicin dose even to patients with a bilirubin level of 3 mg/dl, because we used the less toxic compound epirubicin; furthermore, the dosage of epirubicin scheduled in our protocol for all patients was 40 mg/m^2 and it was given every 4 weeks, so that the dose of anthracycline we used appears superimposable to the half-dose used by Benjamin for patients with bilirubin levels up to 3 mg/dl.

As for toxicity, our treatment caused a significant but bearable toxicity, especially on leucopoiesis. However, the use of haemopoietic growth factors allowed recovery in time for the next cycle even in the patients who experienced grade-III leucopenia on day 15.

The spectrum of non-haematological toxicity was mild and included known manifestations such as alopecia, nausea and mucositis. Hepatic toxicity was difficult to assess in our series, since all patients presented with impaired baseline hepatic function; however, these parameters usually worsened only transiently during treatment, probably as a consequence of the low dosage of epirubicin we used; furthermore, adequate supportive therapy helped minimise hepatotoxicity, which never caused acute liver failure nor treatment delay or discontinuation.

As far as cardiotoxicity was concerned, the maximum cumulative dose of epirubicin allowed in this protocol (400 mg/m^2) does not exceed the dose considered critical for cardiotoxicity [28]. However, 1 patient with no major cardiac alerations at study enrolment experienced a reduction of left ventricular fraction after 9 cycles and a cumulative dose of only 360 mg/m^2.

In conclusion, this treatment appears to be an active and tolerable therapeutic option for HCC patients who are not candidates for surgical or locoregional procedures. In our opinion, a randomised-controlled trial versus best supportive care could probably be useful to elucidate the exact impact of this treatment on patients' survival and quality of life. Thus, even though our phase-II study did not include survival as an end point, we believe that this protocol could

increase unresectable HCC patients life expectancy. Furthermore, the modulation of resistance to anthracycline by means of multidrug-resistance-reverting agents, e.g. verapamil, quinidine, and others [29, 30], may further increase the efficacy of this treatment.

1. Saracci R, Repetto F. Time trends of liver cancer. *J Natl Cancer Inst* 1980, **65**, 241–247.
2. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1996. *CA Cancer J Clin* 1996, **65**, 5–27.
3. Tobe T, Arii S. Improving survival after resection of hepatocellular carcinoma: characteristics and current status of surgical treatment of primary liver cancer in Japan. In Tobe T, Kameda H, Okudaira M, *et al.*, eds. *Primary Liver Cancer in Japan*. Springer-Verlag, Tokyo, 1993, 215–220.
4. Nagorney DM, Adson MA. Major hepatic resections for hepatoma in the West. In Wanebo HJ, ed. *Hepatic and Biliary Cancer*. Marcel Dekker Inc., New York, 1987, 167–185.
5. Livraghi T, Bolondi L, Buscarini L, *et al.* No treatment, resection and ethanol injection in hepatocellular carcinoma: a retrospective analysis of survival in 391 patients with cirrhosis. *J Hepatol* 1995, **22**, 522–526.
6. Yamada R, Sato M, Kawabata T, *et al.* Hepatic artery embolization in 120 patients with unresectable hepatoma. *Radiology* 1983, **148**, 397–401.
7. Williams R, Rizzi P. Treating small hepatocellular carcinomas. *N Engl J Med* 1996, **334**, 728–729.
8. The Liver Study Group of Japan. *Primary Liver Cancer in Japan* (ninth report), 1990.
9. Okazaki N, Okada S, Nose H *et al.* Systemic chemotherapy for hepatocellular carcinoma. In Tobe T, Kameda H, Okudaira M, *et al.*, eds. *Primary Liver Cancer in Japan*. Springer-Verlag, Tokyo, 1993, 301–305.
10. Nerenstone SR, Ihde DC, Friedman MA. Clinical trials in primary hepatocellular carcinoma: current status and future directions. *Cancer Treat Rev* 1988, **15**, 1–31.
11. Cavalli F, Tschopp L, Gerber A, Sonntag RW, Rittsel HJ, Brunner KW. Therapiersultate mit VP 16.213 allein oder kombiniert mit 5-fluorouracil beim leberzell karzinom (hepatoma). *Schweiz Med Wochenschr* 1977, **107**, 1960–1964.
12. Melia WM, Johnson PJ, Williams R. Induction of remission in hepatocellular carcinoma. A comparison of VP-16 with adriamycin. *Cancer* 1983, **51**, 206–210.
13. Oken MM, Creech RH, Tormey DC, *et al.* Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982, **5**, 649–655.
14. Okuda K, Ohtsuki T, Obata H, *et al.* Natural history of hepatocellular carcinoma and prognosis in relation to treatment: study of 850 patients. *Cancer* 1985, **56**, 918–928.
15. World Health Organization. *WHO Handbook for Reporting Results of Cancer Treatment*. Offset publication 48. World Heatlh Organization, Geneva, 1979.
16. Berman MM, Libbey NP, Foster JH. Hepatocellular carcinoma: polygonal cell type with fibrous stroma—an atypical variant with a favorable prognosis. *Cancer* 1980, **46**, 1448–1455.
17. Veenhof CHN. Chemotherapy; options and possibilities. In Lygidakis NJ, Tytgate GN, eds. *Hepatobiliary and Pancreatic Malignancies: Diagnosis, Medical and Surgical Management*. Thieme, New York, 1989, 403–409.
18. Lokich J. Chemotherapy for hepatoma. In Wanebo HJ, eds. *Hepatic and Biliary Cancer*. Marcel Dekker Inc., New York, 1987, 239–253.
19. Porta C, Moroni M, Nastasi G, Arcangeli G. 5-fluorouracil and *d, l*-leucovorin calcium are active to treat unresectable hepatocellular carcinoma patients: preliminary results of a phase II study. *Oncology* 1995, **52**, 487–491.
20. Olweny CLM, Toya T, Katongole-Mbidde E, *et al.* Treatment of hepatocellular carcinoma with adriamycin. Preliminary communication. *Cancer* 1975, **36**, 1250–1257.
21. Barbare J, Ballet F, Petit J, Poupon-Darnis F. Carcinoma hepatocellulaire sur cirrhose: traitement par la doxorubicine. Essay phase I. *Bull Cancer* 1984, **71**, 442–445.
22. Park JG, Lee SH, Hong IG, *et al.* MDR1 gene expression: its effect on drug resistance to doxorubicin in human hepatocellular carcinoma cell lines. *J Natl Cancer Inst* 1994, **86**, 700–705.
23. Goldstein LJ, Galski H, Fojo A, *et al.* Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst* 1989, **81**, 116–124.
24. Giaccone G, Bonardi G, Leria G, Donadio M, Calciati A. Long-term survival and complete response to adriamycin and etoposide in a case of hepatocellular carcinoma. *Tumori* 1986, **72**, 409–411.
25. Van Eeden H, Falkson G, Burger W, *et al.* 5-Fluorouracil and leucovorin in hepatocellular carcinoma. *Ann Oncol* 1992, **3**, 404–405.
26. Benjamin RS, Wiernik PH, Bachur NR. Adriamycin chemotherapy—efficacy, safety and pharmacologic basis of an intermittent single high-dosage schedule. *Cancer* 1974, **33**, 19–27.
27. Johnson PJ, Alexopoulos A, Johnson RD, Williams R. Significance of serum bilirubin levels in response of hepatocellular carcinoma to doxorubicin. *J Hepatol* 1986, **3**, 149–153.
28. Epirubicin. In: Dorr RT, Von Hoff DD. *Cancer Chemotherapy Handbook*. Norwalk, Connecticut, Appleton & Lange, 1994, 434–439.
29. Goldstein LJ. Clinical reversal of drug resistance. *Curr Probl Cancer* 1995, **19**, 65–124.
30. Raderer M, Scheithauer W. Clinical trials of agents that reverse multidrug resistance. A literature review. *Cancer* 1993, **72**, 3553–3563.