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Treatment of Unresectable Hepatocellular Carcinoma With a Combination of Human Recombinant α-2b Interferon and Doxorubicin: Results of a Pilot Study

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Based on the *in vitro* and *in vivo* potentiation of the cytotoxic activity of chemotherapeutic agents by the interferons, a pilot study combining human recombinant α-2b interferon (IFN) and doxorubicin was conducted for the treatment of unresectable, histologically proven hepatocellular carcinoma. Between March 1988 and May 1990, 21 patients (median age: 60 years, range: 29–76) entered the study. The dose of doxorubicin was fixed at 35 mg/m², every 3 weeks. The dose of α-2b IFN was 6 million U/m² per day, 5 days a week. 3 patients (14%) obtained a partial response lasting 11, 16 and 30 months, and 1 had a stable disease during 8 months. The other 17 patients died within a median survival time of 4 months. All patients experienced flu-like symptoms. 7 patients experienced WHO grade III-IV haematological toxicity. We conclude that the association of α-2b IFN and doxorubicin is feasible, with respect to the use of doxorubicin at an inferior dose level than the same agent used without IFN. The response rate is comparable to that observed with doxorubicin used alone. Further phase I studies and randomised trials are required to confirm the role of this regimen in the treatment of unresectable hepatocellular carcinoma.

Key words: hepatocellular carcinoma, doxorubicin, interferon
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INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) generally has a very poor prognosis because it is usually present at an advanced stage [1]. Surgical resection offers the best hope of cure [1]. Even when the tumour is resectable, an operative morbidity rate between 11% and 25% is reported [1–4] and then, the mean 5-year survival rate after complete resection is about 25%. Only a few patients have, in fact, resectable tumours because, in most cases, tumours involve the two hepatic lobes. Some patients have initially metastatic diseases. Moreover, most patients have liver

cirrhosis and cannot be surgically cured because of this underlying disease. For unresectable tumours, only doxorubicin has demonstrated antitumour activity. When this drug is employed at conventional doses, the response rate is about 20%. At the present time, doxorubicin alone is currently the first recommended treatment for HCC [5–7].

The antitumour activity of interferon (IFN) has been investigated in *in vitro* studies. Desmyter and colleagues have shown, in a study using PLC/PRF/5 human hepatoma cell line, that exogenous human leucocyte or human fibroblast IFN had a cellular inhibitory effect [8].

In man, interferons have recently demonstrated antitumour activity in some human tumours [9–11], and moreover, recent studies suggest that the combination of cytotoxic agents and interferons may have synergistic antitumour activity *in vitro* [12–14] and *in vivo* [15, 16].

Among the cytotoxic agents, studies using the clonogenic assay have documented cytotoxic potentiation from the combination of recombinant leucocyte α-IFN and doxorubicin in human tumour cell lines [15–19]. Creagan and colleagues [20] reported in 1989 a phase I–II study in which 26 patients with various, advanced, solid tumours received a combination of recombinant α-IFN (12×10^6 U/m^2), daily for 5 days every week, plus doxorubicin (25–40 mg/m^2 on day 3). A striking partial response during 11.5 months was observed in 1 patient with unresectable HCC.

Because of these data, and considering the *in vitro* and *in vivo* studies suggesting antitumour synergistic activity of the combination of IFN and doxorubicin, a pilot study was undertaken in our department, with the aim of determining the safety and the efficacy of this combination in unresectable, untreated and histologically proven HCC.

PATIENTS AND METHODS

Patients

21 patients with biopsy proven, unresectable and untreated HCC were consecutively assigned non-randomly to receive intravenous doxorubicin and IFN. All the patients were free of previous treatment (no surgical resection, no chemotherapy or radiotherapy, and no tumour embolisation).

Eligibility criteria included an ECOG performance status of less than or equal to three. Before entering the study, patients had a complete clinical evaluation of their tumours by physical examination, a determination of the α-fetoprotein level (α-FP) by enzyme-linked immunoassay (Abbott Laboratories, Chicago, Illinois, U.S.A.), and a complete imaging procedure including CT scan, ultrasonography, chest roentgenography and bone scintigramme. The tumour mass was estimated according to the World Health Organization data [21].

The physical condition of the patients was examined with the usual biochemical profiles, complete blood count and renal and hepatic functions tests. Measurements of the tumour mass were reported after the third treatment, then at regular intervals during follow-up. Even when hepatic function tests were abnormal, impairment was not severe enough to exclude these patients from the trial.

Hepatitis B surface antigen (HBs Ag) and antibodies against HBs Ag were detected by immunoradiometric-assay using kits from Abbott Laboratories.

Patients' characteristics are summarised in Table 1. The 21 patients (male/female 19/2) had a median age of 60 years (range: 29–76). All patients, except 5, had a background of chronic liver disease: alcoholic cirrhosis, $n = 7$; postviral B hepatitis cirrhosis, $n = 5$; alcoholic cirrhosis and postviral B hepatitis, $n = 1$; haemochromatosis, $n = 2$; chronic active hepatitis, $n = 1$.

2 patients (patient numbers 8 and 16) apparently had a single but inoperable tumour. The other patients had multiple hepatic tumour foci, 3 of them with metastasis (peritoneal, pleural and lung involvement for patient numbers 1, 13 and 18, respectively). 4 patients had histologically involved ascites.

2 patients had a normal level of α-FP. 19 patients had a level of α-FP over 5 ng/ml (median level 390; range 6–200 000; normal level < 5 ng/ml).

13 patients had a normal level of bilirubin (normal level < 17 μm/l), and 8 patients had a high level of bilirubin (median 32 μm/l; range 26–108). 17 patients had a high level of alkaline phosphatase (median 216; range 142–700, normal level < 130 μm/ml).

The median delay between diagnosis and treatment was 20 days, with a range from 1 to 297 days.

Treatment

Doxorubicin was given intravenously at 3-week intervals. The initial dose was 35 mg/m^2. The doses of doxorubicin were reduced by one-third if grade 1 haematological toxicity occurred, and by one-half if grade > II haematological toxicity occurred. Doxorubicin was omitted in case of persisting grade III–IV haematological toxicity, and was then administered with adaptation of the dose when the peripheral white blood cell (WBC) count had returned to the normal level. In the case of thrombocytopenia related to the underlying liver disease, we did not make any adaptation of the dose of doxorubicin to the platelets count.

IFN was given subcutaneously or intramuscularly every day, except on Saturday and Sunday, at the daily dose of 6 million U/m^2 per day. This schedule offered more tolerable toxicities than the intravenous administration. The dose of interferon was fixed and not adapted to the WBC count. The injection was preceded by oral administration of paracetamol to prevent the systemic toxic effects (fever, chills) due to utilisation of IFN. Patients were precluded from receiving steroids, non-steroid antiinflammatory drugs, hormones, or other chemotherapeutic drugs.

3. *Criteria of response and toxicities*

We defined objective regressions according to WHO criteria [21]. Duration of response was defined as the time between the beginning of treatment and the diagnosis of progression. Survival time was defined as the time between the day of administration of the first dose of doxorubicin and the time of the patient's death. Toxicity was graded according to WHO data [21].

RESULTS

21 patients entered the study between March 1988 and May 1990. The end point was 31 December 1992.

The 21 patients were evaluable for antitumour response and toxicity. Response to therapy and patient survival are reported in Table 2. One hundred and eight courses of treatment (median

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Table 1. Patients' characteristics

Patient no.	Sex/age	Underlying liver disease	Initial biological data *			Time diagnosis treatment (day)
			α-FP	Bilirubin	Alkaline phosphatase	
1.	M-76†	—	270	7	83	45
2.	M-66	—	2 200	32	262	15
3.	M-54	Postviral B cirrhosis HbS Ag(+)	50 000	56	158	30
4.	M-55	Postviral B cirrhosis HbS Ag(+)	800	7	233	1
5.	M-46	Chronic active hepatitis (post B)	3 500	26	205	195
6.	M-62	Alcoholic cirrhosis	6	17	89	180
7.	M-47	Postviral B cirrhosis	85	18	147	20
8.	F-46‡	—	2 500	12	430	33
9.	M-61	Alcoholic and post B cirrhosis, HbS Ag(+)	530	21	165	9
10.	M-44	Alcoholic cirrhosis	22 000	12	216	60
11.	M-39	Postviral B cirrhosis HbS Ag(+)	27	150	283	30
12.	M-66	Alcoholic cirrhosis	17	46	185	37
13.	F-57†	—	6	6	120	12
14.	M-63	—	120	32	540	10
15.	M-65	Alcoholic cirrhosis	390	11	300	297
16.	M-72‡	Alcoholic cirrhosis	139 000	180	700	6
17.	M-62	Alcoholic cirrhosis	51	10	142	15
18.	M-29†	Postviral B cirrhosis HbS Ag(+)	200 000	103	750	17
19.	M-64	Haemochromatosis	3	17	340	22
20.	M-60	Haemochromatosis	1	20	180	12
21.	M-76	Alcoholic cirrhosis	15 000	17	100	15

*Normal levels: α-FP < 5 ng/ml; bilirubin: < 17 μm/l; alkaline phosphatase < 130 μm/l. †These 3 patients had metastatic disease (peritoneum, pleural and lung involvement, respectively). ‡These 2 patients had a single inextirpable tumour; the other had multiple tumours.

Table 2. Response to therapy and survival

Patients	Number of courses	Change in α-FP level*	Response (months)†	Status‡	Survival (months)§
1	5	270 rose to 17 000	PD	D	5
2	4	2 200 rose to 2 600	PD	D	6.5
3	4	50 000 rose to 125 000	PD	D	8.5
4	5	800 rose to 16 000	PD	D	5
5	1	3 500 rose to 7 000	PD	D	1.5
6	9	Stable level	SD (8)	D	24
7	5	85 rose to 300	PD	D	4
8	2	2 500 fell to 2 040	PD	D	1.5
9	2	530 rose to 800	PD	D	1.5
10	7	22 000 rose to 42 000	PD	D	7
11	1	27 fell to 9	PD	D	1
12	2	17 fell to 11	PD	D	2.5
13	3	6 fell to 4	PD	D	3
14	3	120 rose to 487	PD	D	5
15	3	390 fell to 320	PD	D	3.5
16	16	139 000 fell to 2 600	PR (16)	D	26
17	1	51 fell to 46	PD	D	1
18	1	200 000 rose to 2 000 000	PD	D	0.5
19	11	Stable level	PR (11)	D	44
20	5	Stable level	PD	D	4
21	30	15 000 fell to 4·5	PR (30)	A	34+

*Between the start of therapy and the latest administered treatment. †PR: partial response; PD: progressive disease; SD: stable disease. ‡D: dead; A: alive. §From start of treatment.

5; range 1–30) were administered to these 21 patients. 4 patients received only the first course of therapy and rapidly died from the progression of their disease.

The overall median survival time was 4 months (range 0.5–44 months). 17 patients had rapidly progressive disease and died of their HCC, with a median duration of survival of 4 months (range: 0.5–8.5 months). Among the other 4 patients without evidence of initial failure to therapy, 1 patient (number 6) with fibrolamellar hepatocarcinoma developed alcoholic cirrhosis, received nine courses of treatment, had a stable disease of 8

months and died after a total survival of 24 months. 3 patients (14%) obtained a partial response (PR). Among them, the first patient (number 16) had a single but inoperable tumour, developed against a background of alcoholic cirrhosis. He had a PR of 16 months with a > 50% regression of the initial tumour mass (Figure 1a, b) and a decrease of 98% of the initial level of α-FP, from 139 000 to 2 600 ng/ml. Figure 2(a) shows the initial evolution of the serum α-FP level and of the serum alkaline phosphatase level. The rapid decrease of the serum α-FP level occurred during the fifth course of treatment. This was apparently due to massive tumour necrosis with, at the same time, an abrupt rise of the bilirubin and of the serum alkaline phosphatase levels. This patient had, at that time, a septicaemia due to *Campylobacter jejuni* without documented infection of the ascite. This patient died of gastric haemorrhage after a total duration of survival of 26 months.

The second responder patient (number 19) had multiple hepatic tumour foci developed against a background of haemochromatosis. He had a PR according to the measurement of the tumour mass after eleven courses of therapy. The serum α-FP level was normal at the beginning of the treatment. The duration of the response was 11 months. The treatment was unfortunately stopped because of a non-tolerable flu-like syndrome and particularly a depressive syndrome, both related to IFN administration. This patient died of progressive disease after a total duration of survival of 44 months.

The third responder patient (number 21), who had a bifocal tumour mass developed on a background of alcoholic cirrhosis, obtained a PR of 30 months according to the measurement of the tumour mass. However, according to the measurement of his serum α-FP level, this patient obtained a complete biological response with a fall of serum α-FP level from 15 000 ng/ml at the beginning of the treatment to 4.5 ng/ml at the third course (Figure 2b). This decrease occurred principally in the first month of treatment with, as for the first patient, an episode of gram negative organisms (*E. coli* and *Klebsiella*) septicaemia, without documented infection of the ascite, while he was in aplasia. This patient is alive with progressive disease at 34+ months.

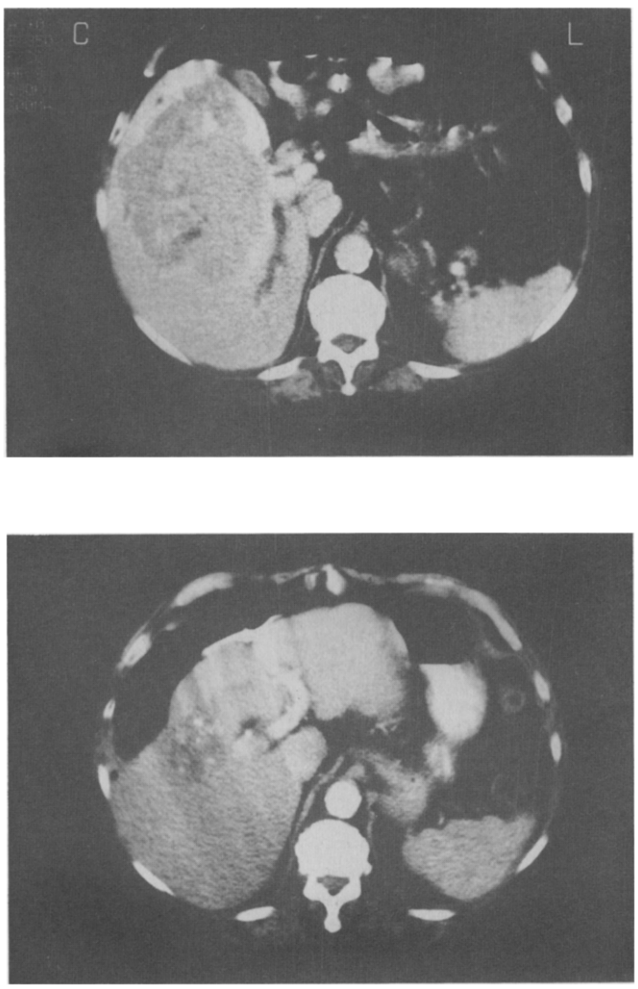

Figure 1. (a) Patient number 16: initial dynamic CT. Heterogenous postcontrast enhancement of a large hypervascularised hepatocellular carcinoma, with compression of the bile ducts, but without thrombosis of the portal vein. (b) Patient number 16: dynamic CT 6 months after treatment. Clear modifications of this tumour with intense decrease in size, hypodense aspect, and regression of the biliary ducts dilatation.

Toxicity

The overall toxicity is reported in Table 3.

All the patients experienced at least one episode of fever and/or weakness due to the IFN-administration, in spite of preventive paracetamol administration. However, this treatment was generally well tolerated, and only 1 patient (number 11) required discontinuation of the therapy after his eleventh course because of intolerance to the IFN-administraton (flu-like syndrome and depressive manifestations).

As expected, the main toxicity was haematological. 10 patients (48%) had at least one episode of toxicity according to the level of polymorphonuclear neutrophils. 1 patient had grade I haematological toxicity, 2 patients had grade II toxicity, 6 patients had grade III toxicity, and 1 patient had an episode of grade IV toxicity. These toxicities were always reversible and did not occur in following courses with the adjustment of the dose of doxorubicin according to the protocol treatment.

Thrombocytopenia due to the IFN–doxorubicin administration is reported in Table 3. This toxicity was never complicated by haemorrhagic phenomena, and never required platelet transfusions.

We did not observe other IFN-related complications, and particularly, we did not have any major hepatotoxicity due to the concurrent administration of doxorubicin and IFN. Finally, we did not observe cardiotoxicity due to the doxorubicin administration, nor renal toxicity due to IFN.

DISCUSSION

We have described here a therapeutic scheme based on the potentiation of doxorubicin therapy by the concurrent administration of α-2b-IFN for unresectable HCC. The efficacy of IFN has been well documented in some haematological diseases such as hairy cell leukaemia, myeloma or lymphomas. IFN also has therapeutic effects in some solid tumours such as melanoma [22] or renal cell carcinoma [23].

Synergism between IFN and chemotherapeutic agents has been documented with a few drugs like vinblastin [24], dacarbazine [25] and more recently with doxorubicin. Balkwill and Moodie [26] found that human α-IFN (of lymphoid origin) plus doxorubicin was synergistic against human breast cancer growing in nude mice. The majority of the *in vitro* and *in vivo* data provoked interest in a clinical trial with an α-IFN plus doxorubicin regimen. Aapro and colleagues [18] have demonstrated additive effects of recombinant α-IFN against myeloma, breast and colon lines. Welander and colleagues [27] have

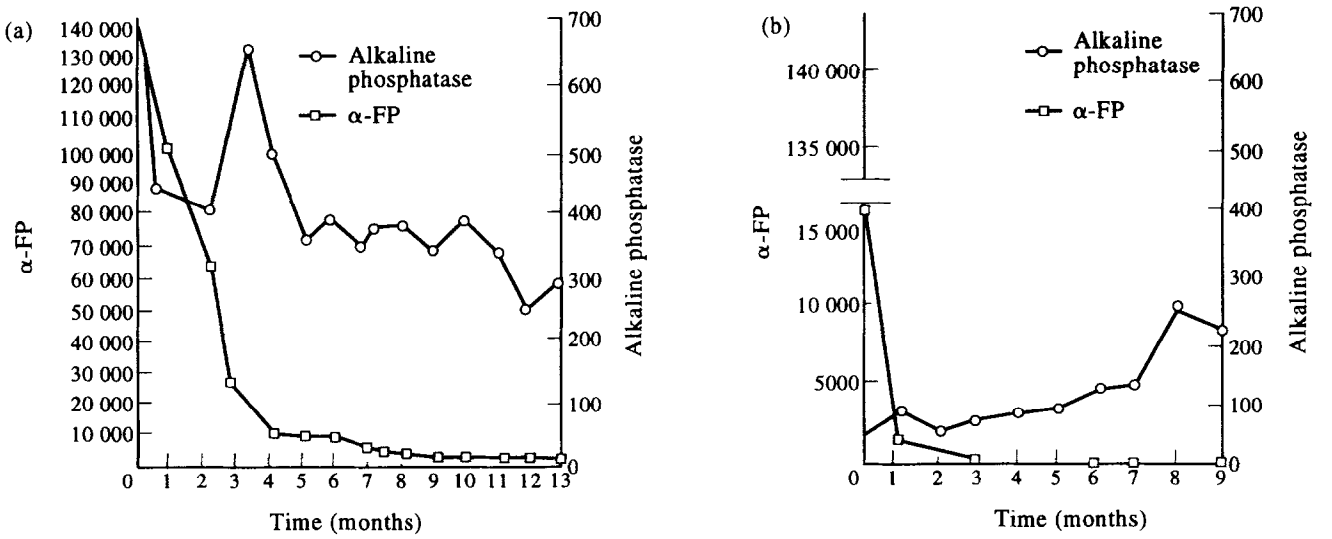

Figure 2. (a) Initial evolution of α-FP and alkaline phosphatase levels in patient number 16. (Normal levels: α-FP < 5 ng/ml; alkaline phosphatase < 130 μm/l.) (b) Initial evolution of α-FP and alkaline phosphatase levels in patient number 21.

Table 3. Toxicity of the treatment

Type of toxic effect		Frequency or number of patients
1. General symptoms		
Nausea		All patients had at least one episode of nausea due to doxorubicin administration
Alopecia		All patients (WHO grade IV for all patients)
Fever, weakness, and flu-like symptoms due to IFN.		All patients, 1 of these requiring the interruption of the treatment
2. Haematological toxicity		
Neutropenia (grade WHO)	I	1 patient
	II	2 patients
	III	6 patients
	IV	1 patient
Thrombocytopenia (grade OMS)	I	2 patients
	II	3 patients
	III	2 patients
	IV	1 patient
3. Other toxicities		
Hepatic, cardiac or renal toxicity		None
Infection		2 patients (with documented gram-negative organisms septicaemia)
Neurological		1 patient (depressive manifestations)

documented true synergism of recombinant α-2b IFN plus doxorubicin against several human tumour cell lines growing *in vitro*. Von Hoff and colleagues [19] have reported the results of 13 *in vitro* studies combining α-2b IFN and doxorubicin; nine showed synergistic activity.

For HCC, in the absence of the possibility of total surgical resection, there is actually no curative therapy. The use of doxorubicin in the first documented treatment by Olweny [28] gave an unexpectedly high response rate (11 out of 14 patients). Subsequent attempts to confirm this high response rate have been unsuccessful and most studies report an 11 to 25% response rate [29]. Nevertheless, doxorubicin alone is currently the first recommended treatment for these patients.

Some authors consider that doxorubicin is not ideal for the treatment of unresectable HCC because of the toxicity of this drug. In a prospective randomised trial (doxorubicin versus no therapy), Lai and colleagues [30] reported that doxorubicin caused fatal complications (septicaemia or cardiotoxicity) in 25% of the patients. The therapeutic result was significantly in favour of the doxorubicin group in terms of median survival time (10.6 weeks versus 7.5 weeks, $P = 0.036$), but it is clear that this result is of little benefit to the survival (3.1 weeks).

In the absence of other effective drugs in HCC, and according to the potentiation of doxorubicin by IFN administration in many solid tumours [31, 32], it was a logical consideration to associate IFN and doxorubicin for unresectable HCC in a prospective non-randomised study. Since continuous exposure to recombinant α-2b IFN produces more cytotoxicity than short-time infusion, it was decided to administer IFN every day, except on Saturday and Sunday [19]. With this combination, Creagan and colleagues obtained a striking partial response lasting 11.5 months in a patient with HCC associated with an α-FP reduction from 39 000 ng/ml to 299 ng/ml [20].

In our study, 3/21 evaluable patients obtained a PR (of 11, 16 and 30 months). The response rate was 14%. This response rate observed in our study is similar to that generally reported when doxorubicin is used alone at a dose of 60 to 75 mg/m^2 every 3 weeks, generally with brief response durations [7, 29, 33, 34]. However, it is very important to note that two of the responses

were dramatic, with an abrupt fall of the α-FP level associated with documented tumour necrosis and, in both of the patients, a gram negative organism septicaemia. These 2 patients had particularly durable partial responses (16 and 30 months). However, the median survival time observed in this study is rather poor; this may be due to the fact that recruitment of patients was independent of their life expectancy.

In fact, the role of IFN remains unclear in HCC. Sachs and colleagues [35] have treated 16 patients with unresectable HCC by recombinant leucocyte α-IFN (12–50 million U/m^2), three times a week for 12 weeks. Only 2 patients completed the entire 12-week course: 1 had a stable disease, the other had a progressive disease. Most patients died from disease progression, but in some, toxic side effects to IFN played a role. The mean survival time for the 13 patients who died was 7.9 weeks. For the authors, the results were disappointing and the toxicity of the treatment too high to be acceptable. Lai and colleagues [36] more recently conducted a second study in which they compared, in a prospective randomised trial, doxorubicin with α-2 IFN for inoperable HCC. 75 Chinese HCC entered the study, 25 patients received doxorubicin, 50 were randomised to receive α-2 IFN, either 18 million U/m^2/day, or 50 million U/m^2/day, intramuscularly 3 times a week. The two groups receiving α-2 IFN were grouped together for treatment evaluation. The median survival was 4.8 weeks for the doxorubicin group and 8.3 weeks for the IFN group, with a significantly better efficacy in terms of response rate in the IFN group. For these authors, α-2 IFN was superior to doxorubicin for inoperable HCC. In 1993, they also reported a randomised controlled trial, in which 71 chinese patients were randomly assigned to receive recombinant α-2a IFN (50 million U/m^2, three times a week) or no antitumour therapy. The response rate was 31.4% (11 patients of 35 who received IFN). The survival of patients treated with high-dose IFN was significantly better (median survival time: 14.5 weeks versus 7.5) than that of patients who received no therapy (P = 0.0471) [37]. For the GITSG (Gastrointestinal Tumour Study Group), IFN had no efficiency in the treatment of HCC, with a response rate of only 7% (38).

Recently, Kardinal and colleagues reported the results of a study in which 31 eligible patients received a therapeutic regimen similar to our protocol. The dose of doxorubicin was 25–40 mg/m^2 given intravenously on day 3, and that of IFN was 12 × 10^6 units/m^2/day for 5 days given by intramuscular injection (both repeated every 4 weeks). Haematological and non-haematological toxicities were significant but tolerable; the response rate was 3% and the median survival for all patients was 10 months. The authors did not recommend this combination. However, although the dose of doxorubicin was similar, the total dose of IFN was inferior to that administered in our therapeutic regimen. This unusual schedule could be detrimental to the results [39].

Carr and colleagues have proposed a different approach, using aggressive intra-arterial chemotherapy, combining doxorubicin and cisdiamine dichloroplatin every 4 weeks, with α-IFN (3 million units, given subcutaneously, three times a week). Among 25 evaluable patients who received three courses of therapy, 15 (60%) obtained a partial response. 9 patients subsequently underwent orthoptic liver transplant [40].

In our study, the main toxicity, besides the flu-like syndrome, was, as expected, haematologic. 7 patients (33%) experienced grade III or IV neutropenia. Creagan and colleagues [20] reported a maximum tolerated dose of doxorubicin of 40 mg/m^2 when used in combination with IFN. For Sarosy [41], the haematologic toxicity occurred in 50% of patients treated with doxorubicin at 40 mg/m^2 every 3 weeks plus IFN (10 × 10^6 U/m^2, subcutaneous, three times a week), and in 25% of patients treated with doxorubicin at 30 mg/m^2. The therapeutic scheme reported by Green [31] is somewhat different, and for the authors, the maximum tolerated dose of doxorubicin was 25 mg/m^2 every week for 3 weeks, when administered with IFN, 10 million U/m^2, subcutaneously, three times a week then followed by 2 weeks of rest. We think that the optimal dose of doxorubicin when administered with IFN is approximately 40 to 50% of the usual dose of doxorubicin when administered as a single agent. For Kardinal and colleagues the maximum dose of doxorubicin that could be administered with IFN was only 40 mg/m^2 every 4 weeks [39].

The other toxicities related to the use of IFN are comparable to other studies. All patients experienced at least the flu-like symptoms of chills, fever and muscle aches. Since these systemic symptoms were reversible and tolerable, none of these was considered dose limiting. This toxicity could however be very important and lead to the discontinuation of the treatment. One patient who had obtained a PR, required a discontinuation of the treatment because of intolerable flu-like syndrome and particularly a major depressive syndrome, which we know to be a possible toxic effect of IFN administration [42]. We did not observe major hepatotoxicity such as that reported by Green [31] but the therapeutic scheme used in our study was somewhat different, and the total dose of doxorubicin administered every 3 weeks was inferior to that administered by Green [31] at the end of each 3-week period of treatment (35 mg/m^2 in our study versus 75 mg/m^2 in Green's study).

In conclusion, the potential of this association remains unclear. We think that this treatment is well tolerated and could be safely administered in this type of patient. The toxicity is generally moderate. The response rate is comparable to that reported with doxorubicin used alone in HCC. Further phase I trials using different doses of IFN in combination with a fixed dose of doxorubicin could be proposed. However, randomised studies comparing IFN plus doxorubicin versus doxorubicin alone have to be made in the treatment of unresectable HCC, and other chemotherapeutic combinations, possibly via hepatic artery and in association with IFN given at high dose, must be tested.

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