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Low-dose Interleukin-2 Subcutaneous Immunotherapy in Association with the Pineal Hormone Melatonin as a First-line Therapy in Locally Advanced or Metastatic Hepatocellular Carcinoma

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Experimental studies have showed that hepatocellular carcinoma (HCC) cells are susceptible to cytolysis of interleukin (IL)-2-activated lymphocytes. Moreover, our previous studies demonstrated that the pineal neurohormone melatonin (MLT) may enhance IL-2 efficacy. On this basis, a study was started with low-dose IL-2 (3 million U/day subcutaneously for 6 days/week for 4 weeks) plus MLT (50 mg/day orally every day given in the evening) as a first-line therapy of unresectable HCC. The study included 14 patients. Objective tumour regressions were obtained in 5/14 (36%) patients (one complete response, four partial responses), with a median duration of 7+ months. 6 patients had stable disease, while the other 3 progressed. Toxicity was low in all cases. This study shows that the neuroimmunotherapy with low-dose IL-2 plus MLT is a new well-tolerated and effective therapy of advanced HCC.

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INTRODUCTION

THE ADVANCED hepatocellular carcinoma (HCC) still remains an untreatable disease. When surgery is possible, long-term control of the disease can be achieved. In contrast, the prognosis of advanced unresectable HCC is poor and the median survival time is generally less than 1 year. There is also evidence that there is a difference in the prognosis between those liver neoplasms which produce large amounts of alpha feto-protein (AFP) and those that do not [1].

A large number of chemotherapeutic agents have been used in the treatment of advanced HCC, without, however, any clear benefit on survival. In fact, randomised clinical trials have showed no advantage for intra-arterial versus systemic chemotherapy versus supportive care alone [2]. The response rate to the different chemotherapeutic combinations is generally less than 20% [2]. Moreover, the endocrine strategies also seem to be less effective in advanced HCC [3].

Biological response modifiers (BRM) could constitute new possible therapeutic approaches in the treatment of HCC. Within the BRM group, immunotherapy with interleukin-2 (IL-2) would represent the most promising strategy, because of its capacity to activate a biologically effective antitumour immune response [4]. Cells from primary liver carcinomas have been shown to be susceptible to cytolysis by IL-2-activated cytotoxic lymphocytes [5]. Preliminary clinical investigations with IL-2, administered via the hepatic artery, would suggest potential antitumour activity of IL-2 in HCC [6, 7]. At present, however, the clinical efficacy of a systemic immunotherapy with IL-2 in HCC still has to be established. Very preliminary results with high-dose IL-2 intravenous infusion showed no efficacy in a small number of patients with HCC [8].

Our previous clinical results have suggested that the immunomodulating pineal hormone melatonin (MLT) may enhance IL-2 biological and clinical activity [9], through possible inhibition of macrophage-mediated immunosuppressive events, which occur during IL-2 immunotherapy concomitant with the generation of an anticancer immune response [10].

On this basis, we have designed a pilot study with low-dose IL-2 plus MLT as a first-line therapy for locally advanced or metastatic HCC.

MATERIALS AND METHODS

From May 1991 to November 1992, 14 consecutive patients (12 males, two females, median age 56 years, range 39–67) with locally advanced or metastatic HCC entered the study. Eligibility criteria included histologically proven, unresectable HCC, measurable lesions and no previous systemic chemotherapy. Patients with brain metastases and/or second neoplasms were not included in the study. However, low performance status (PS), as evaluated according to Karnofsky's score, and the evidence of abnormally high bilirubin levels were not considered as criteria of exclusion. The experimental protocol was explained to each patient, and informed consent was obtained. Distant organ metastases were present in 8 patients, while the remaining 6 cases showed a locally advanced disease. Predominant sites of metastases were peritoneum 2, bone 2, lung 4. The peritoneal disease was documented by computer tomography (CT) scan and by the cytologic examination of the peritoneal fluid. No patients had fibrolamellar variants of HCC. Moreover, 8 patients were untreated, 2 relapsed after radical surgery, and the other 4 relapsed after chemoembolisation with doxorubicin. No patient was under treatment with pharmaceuticals which influence the immune system during the study.

Human recombinant IL-2 was supplied by Euro-Cetus (Amsterdam, the Netherlands), and MLT by Medea Research (Milan, Italy). IL-2 was given subcutaneously at a dose of 3 million U/day at 8.00 p.m. for 6 days/week for 4 consecutive weeks, corresponding to one immunotherapeutic cycle. MLT was given orally at a daily dose of 50 mg at 8.00 p.m. every day until the end of the cycle, starting 7 days before the first IL-2 injection. Both IL-2 and MLT were given in the evening because of their potentially greater biological efficacy in this period of the day [9]. In responder patients or in those with stabilisation of the disease, a second cycle of immunotherapy was administered after a 21-day rest period. After that, patients underwent maintenance therapy consisting of 1 week of therapy every month, until progression of the disease. Clinical response and toxicity were evaluated according to WHO criteria. Complete response (CR) was defined as complete resolution of all evaluable disease for at least 1 month; partial response (PR) as at least a 50% reduction in the sum of the products of the longest perpendicular diameters of measurable lesions for at least 1 month; stable disease (SD) as no objective tumour regression or increase greater than 25% and progressive disease (PD) was defined as at least a 25% increase in measurable lesions or the appearance of new lesions. Radiological examinations were repeated after each cycle of therapy, then every 2 months, and tumour regressions were documented by CT scan. The duration of response was calculated from the onset of therapy. Finally, the clinical response was independently and externally reviewed.

Routine laboratory tests were repeated at weekly intervals. The biological response to the immunotherapy was evaluated by determining the increase in lymphocyte and eosinophil numbers, as demonstrated previously [11,12]. Moreover, to determine macrophage-mediated suppressive events, we measured at 1-week intervals serum levels of neopterin [10] by radioimmunoassay (RIA) with commercially available kits (Henning, Berlin, Germany). The biological data were analysed without the knowledge of the clinical response. AFP serum concentrations were detected before and at the end of each cycle, by using RIA commercial kits (Sorin, Saluggia, Italy). Normal values obtained in our laboratory (95% confidence limits) for AFP were less than 10 U/ml. Data were statistically analysed by the χ^2 test, the Student's t-test and analysis of variance, as appropriate.

RESULTS

Individual clinical data and response to therapy are reported in Table 1. Abnormally high pretreatment values of AFP were found in 7 patients, while they were within the normal range in the other 7 cases. Moreover, 2/14 patients showed high levels of bilirubin before therapy. CR was achieved in 1/14 (7%) patients (duration 13+ months). PR was obtained in 4/14 (29%) patients (duration 22+, 7+, 4+ and 3+ months). Therefore, the overall response rate was 5/14 (36%) patients, with a median duration of 7+ months (range 3+–22+). The responder patient with bone metastasis showed a regression of greater than 50% of the primary tumour and a stabilisation of the bone lesion. 6 other patients (43%) obtained SD, while the remaining 3/14 (21%) progressed rapidly during the first cycle. No significant differ-

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Table 1. Individual clinical data and response to the immunotherapy with IL-2 plus MLT in 14 patients with advanced HCC

Patients	Sex	Age	PS Before	PS After	AFP (U/ml)	Cancer cirrhosis	Metastatic lesions	Clinical response	Response duration (months)	Survival time (months)
1	M	54	50	90	7	No	Peritoneum	PR	22+	22+
2	M	60	40	40	6	No	Lung	PD	—	4
3	F	44	80	100	2	No	—	CR	13+	13+
4	M	56	40	50	8	Yes	Bone	SD	3	6
5	F	52	50	80	1278	No	Bone	PR	7+	7+
6	M	54	10	20	5	Yes	Lung, bone	PD	—	3
7	M	58	50	60	986	No	Lung	PR	4	9
8	M	61	20	30	6	No	Peritoneum	PD	—	3
9	M	67	90	90	41 000	No	—	SD	6+	6+
10	M	51	80	80	23	Yes	—	SD	5+	5+
11	M	58	40	60	5	Yes	—	SD	4+	4+
12	M	69	70	80	1070	No	—	PR	3+	3+
13	M	39	60	60	544	No	Lung	SD	3+	3+
14	M	62	90	90	1400	No	—	SD	3+	3+

PS, performance status (Karnofsky's score); AFP, alpha feto-protein (normal values: less than 10 U/ml); M, male; F, female; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

ence in response rate was seen between patients with normal or high pretreatment values of AFP (2/7 versus 3/7). Moreover, the response rate observed in patients with metastatic disease was not significantly different to that seen in patients with locally advanced neoplasm (3/8 versus 2/6). On the contrary, the response rate was significantly higher in patients who had no concomitant cirrhosis than in those affected by cancer cirrhosis (5/10 versus 0/4, $P < 0.05$). In the same way, the response rate was significantly higher in patients with a PS greater than 40% than in those with a PS of 40% or less (5/9 versus 0/5, $P < 0.01$). Response rates in relation to the main clinical variables are reported in Table 2.

As illustrated in Fig. 1, mean increases in eosinophil and lymphocyte number observed in responder patients were significantly higher than those seen in patients with SD or PD. Conversely, progressed patients showed a mean increase in neopterin levels during the immunotherapy, which was significantly higher than that observed in patients with response or SD, as illustrated in Fig. 2.

Toxicity was low in all patients, and it consisted of fever (greater than 38°C) in 1/14 patients (7%), hypotension grade 1 in 1/14 (7%) patients and thrombocytopenia grade 1 in 2/14 (14%) patients. A decrease in bilirubin levels occurred during treatment in 1 of the 2 patients with high pretreatment values. Hyperpyrexia disappeared during immunotherapy in 1 patient showing

Table 2. Objective tumour regression (CR + PR) rate in relation to the main clinical variables in 14 advanced HCC patients

Characteristic	*n*	CR + PR
Alpha feto-protein		
Normal values	7	2/7 (29%)
High values	7	3/7 (43%)
Concomitant cirrhosis		
No cirrhosis	10	5/10 (50%)*
Cirrhosis	4	0/4 (0%)
Distant organ metastases		
No metastases	6	2/6 (33%)
Metastases	8	3/8 (38%)
Performance status		
> 40	9	5/9 (56%)**
≤ 40	5	0/5 (0%)

* $P < 0.05$ versus patients with cirrhosis; ** $P < 0.01$ versus patients with low PS.

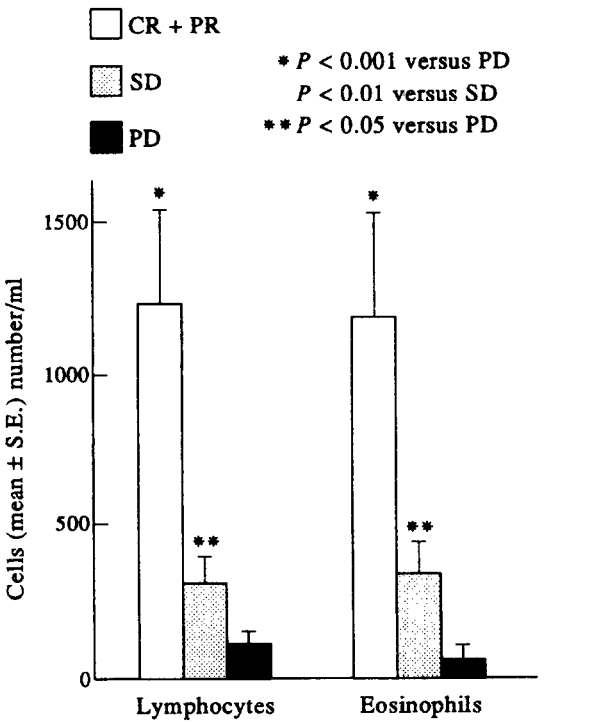

Fig. 1. Increase (mean ± S.E.) in lymphocyte and eosinophil numbers, during the immunotherapy in advanced HCC patients with complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD).

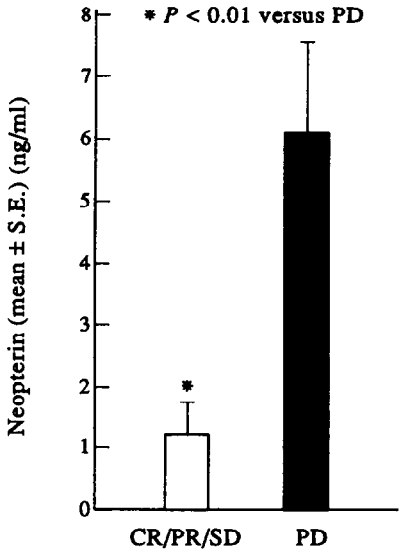

Fig. 2. Increase (mean ± S.E.) in serum levels of neopterin during immunotherapy in advanced HCC patients with complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). There was no significant difference between serum levels of patients with SD, CR or PR.

systemic fever prior to therapy. Finally, a clinically evident improvement of PS was observed in 4/8 (50%) patients with pretreatment values of 50% or less.

DISCUSSION

The results of this phase II pilot study show that low-dose IL-2 is able to induce objective tumour regression in patients with advanced HCC when it is associated with the pineal neurohormone MLT. The tumour regression would seem to be mediated by lymphocyte and eosinophil increases, while the activation of macrophages, as evaluated by determining neopterin increases, appears to be related to a lack of clinical efficacy. Moreover, it should be noted that immunotherapy-induced tumour regression seems to be associated with a longer survival, as suggested by the long duration of response.

Response rate obtained with low-dose IL-2 plus MLT is at least comparable to that reported with chemotherapy [2], but with an apparent better tolerability. In fact, this immunotherapeutic combination appeared to be tolerated even in patients with very low PS, who were unable to undergo conventional chemotherapeutic regimens. However, when PS was very low, the immunotherapy seemed to be generally ineffective in producing tumour regression, and its efficacy was limited to improving the quality of life in some cases. In other words, there may be a threshold of biological damage in cancer patients beyond which the possibility of determining an activation of host defenses against cancer is very limited. In addition to the low PS, another unfavourable prognostic index for a clinical response to immunotherapy in patients with advanced HCC is the concomitant existence of cirrhosis. This finding might be explained by the fact that the fibrosis related to the cirrhotic process would counteract the diffusion of cytotoxic lymphocytes and their infiltration into tumour tissue, resulting in diminished contact between immune and cancer cells, a process which is essential for cancer cell destruction.

In conclusion, this pilot study shows that advanced HCC may be considered as a neoplasm which is responsive to IL-2, when it is associated with a neuroendocrine strategy capable of antagonising macrophage-mediated suppressive events, such as the pineal hormone MLT [9]. Therefore, because of its antitumour efficacy and its good tolerability, even in patients with low PS, this neuroimmunotherapeutic combination may constitute a new promising therapy of advanced HCC.

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