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Immunochemotherapy with Recombinant Interleukin-2 and Adriamycin in Primary Hepatocellular Carcinoma

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SUMMARY **Recombinant interleukin-2 (rIL-2) and adriamycin were administered systemically to treat nine patients (age 15.5-68 years, mean 48.9 ± 15.5 years) with far advanced primary hepatocellular carcinoma. Three patients were newly diagnosed, and the remaining patients had received surgery, transcatheter arterial embolization, chemotherapy and other treatments but without improvement. rIL-2 was given at a dose of 10,000 to 30,000 units/kg every 8 hours for consecutive 9 days, and on the fifth day, a single dose of adriamycin 30 to 60 mg/m^2 was administered. Four patients interrupted the immunotherapy because of severe intolerable side effects, 4 patients completed one course and the remaining one received 2 courses of treatment. Various adverse reactions were encountered, however, they subsided promptly after stopping therapy. All patients failed to respond to the regimen. Primary hepatic tumors continued to enlarge in 8 patients and remained unchanged in one, and pulmonary metastasis also increased in size and number in 4 patients. Transient decrease in serum alpha-fetoprotein was found in 6 patients. These results suggest that systemic IL-2 immunotherapy, even in combination with chemotherapy, is not effective for the treatment of far advanced hepatocellular carcinoma. However, in view of its immune amplifying effect, rIL-2 in combination with other treatment modalities may still be worth trying in early stages of hepatocellular carcinoma.**

Seroepidemiologic surveys conducted in Taiwan have revealed an extraordinarily high prevalence rate of asymptomatic hepatitis B surface antigen (HBsAg) carriers,[1-3] and HBsAg was positive in 82.7% of patients with hepatocellular carcinoma (HCC).[4] The demonstration that HBV DNA was integrated into the host cellular genome strongly supports the causal relationship between chronic HBV infection and occurrence of HCC.[5,6] In Taiwan, primary HCC ranks first among the causes of death from malignancies,[7] and once the diagnosis is established, the prognosis is rather poor except in those with small, excisable tumors.

Recently, several lymphokines, including interleukin-2(IL-2) have been enthusiastically tried in many human viral infections, autoimmune diseases, hematological disorders, allergic diseases and cancers.[8,9] IL-2 injection in mice can stimulate marked *in vivo* proliferation of lymphoid cells in liver, spleen, lungs, kidneys and mesenteric lymph nodes.[10,11] Our previous studies further showed that lymphokine-activated killer (LAK) cells could be generated both *in vivo* and *in vitro* from mononuclear cells of HCC patients, and could lyse fresh autologous and allogeneic and cultured HCC cells.[12,13] Moreover, immunochemotherapy using IL-2 and adriamycin could not only rescue the immunosuppression associated with cytotoxicity of adriamycin but also show synergistic tumoricidal activity in murine studies.[14,15] Therefore, In this study we adapted the therapeutic protocol combining IL-2 and adriamycin in treating 9 patients with far advanced HCC.

MATERIALS AND METHODS

Patients

From September 1987 to October

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1988, 9 HCC patients were admitted to the National Taiwan University Hospital and were treated with rIL-2 and adriamycin. The basic clinical data are summarized in Table 1. The age ranged from 15.5 to 68 years with a mean of 48.9 ± 15.5 years. There were 8 males and one female. The interval between diagnosis of HCC and start of immunochemotherapy ranged from 2 weeks to 16 months. Three patients were newly diagnosed and had not received any specific treatment. The remaining patients had previously been treated with surgery, transcatheter arterial embolization, intra-arterial and systemic chemotherapy, intratumor ethanol injection, and radiotherapy but without evident effect, and only supportive measures were given within recent one month. HBsAg was positive in all 8 patients who had been checked and the serum level of alpha-fetoprotein was markedly elevated in 7 of them. The clinical trial had been approved by the Human Research Committee of this hospital and informed consents were obtained before study.

Lyophilized rIL-2 (Cetus Corporation, Emeryville, California, USA) was dissolved gently into 5% glucose water to make a final concentration of 3×10^6 units/ml and was used within 4 hours after reconstitution. Initially, rIL-2 at a dose of 30,000 units/kg was administered through intravenous bolus or infusion up to 4 hours. The dose of rIL-2 was then adjusted according to the patient's tolerance. rIL-2 was given for consecutive 9 days, and on the fifth day a single dose of adriamycin of 30-60 mg/m^2, based on the serum bilirubin level, was given intravenously.

Acetaminophen and diphenhydramine were given to patients for fever, chills and headache, and metoclopramide was used to control nausea and vomiting. In addition, total parenteral nutrition, blood component transfusion, parenteral antibiotics, colloid and albumin infusion, diuretics, vasopressors and topical emollients were supplied as needed.

Clinical evaluation

Clinical response and adverse effects during and after therapy were closely observed and recorded. Hemogram, liver function, renal function and electrolytes were checked at least twice a week throughout the study. Chest X-rays and EKG were taken if necessary. Image studies including abdominal sonography, computed tomography and hepatic angiography were done to evaluate the changes of the structure and sizes of the tumors after treatment.

Immunological studies

Blood was drawn before treatment, 6th day during treatment (8 hours after IL-2 infusion) and one day after completion of immunotherapy. Mononuclear cells were separated by the density gradient centrifugation of Boyum.[16] The number of natural killer cell were analysed by fluorescence activated cell sorter (FACS 420, Beckton-Dickinson), using Leu 11b (CD16) monoclonal antibody (Becton-Dickinson). LAK activity against J5, an HCC cell line and natural killer cell (NK) activity against K562, a myelogenous leukemia cell line, were determined by a 4-hour ^{51}Cr-release assay.[17]

RESULTS

Clinical responses

A total of 9 patients received rIL-2 and adriamycin treatment. Four patients completed one course of therapy and another one received two courses. Therapy had to be

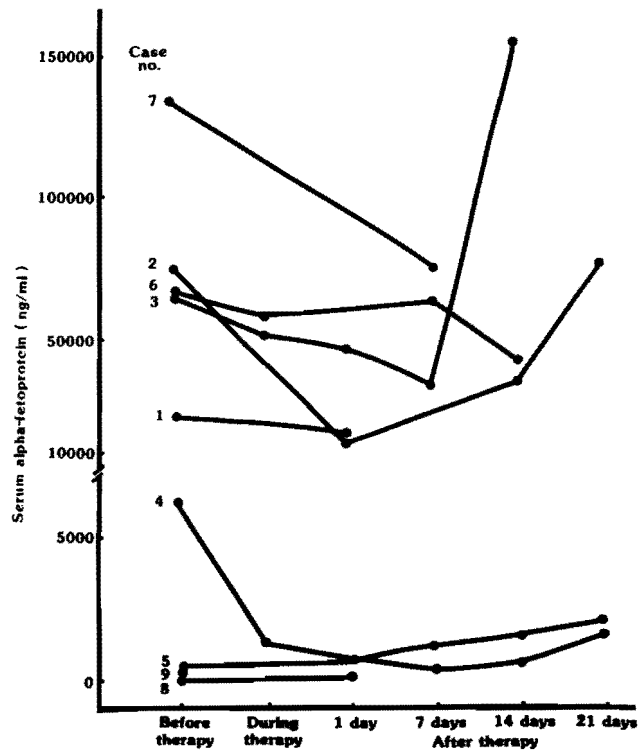

Fig. 1. Changes of serum alpha–fetoprotein in the course of immunochemotherapy with recombinant interleukin–2 and adriamycin.

discontinued before the completion of the 9-day course in the remaining four patients, due to various severe intolerable side effects including hypovolemic shock, dyspnea, abdominal distension, spiking fever, chillness, hallucination, severe diarrhea, oral ulcer and perianal erosion. No death due to the therapy itself was seen.

All patients failed to respond clinically to the regimen. Despite therapy, hepatic tumors continued to progress in 8 patients, as judged by image studies or physical examination, and remained unchanged in one. Moreover, tumor thrombi appeared in the initially empty portal vein in one patient. In the four patients with lung metastasis, the metastatic nodules continued to increase in size and/or number. Serum levels of alpha-fetoprotein decreased obviously in 6 patients, although they usually rebounded promptly after therapy was stopped (Fig. 1).

Adverse reactions

The adverse reactions to rIL-2 and adriamycin therapy are listed in Tables 2 and 3. Shaking chills followed by spiking fever, severe anorexia and general malaise were noted in all patients. Seven patients (78%) suffered from nausea, vomiting and watery diarrhea. These symptoms subsided within a few days after therapy. Productive cough occurred in 5 patients (67%) and interstitial infiltration on chest X-ray was noted in 4 patients (44%). Pleural effusion developed in 5 patients, but 3 of them had had pulmonary metastasis before treatment. Dyspnea and orthopnea occurred in 4 patients (44%), including those two with pulmonary metastasis and pleural effusion. No intubation was needed in these patients.

Generalized edema was seen in 8 patients (89%). Obvious weight gain was noted in 6 (86%) of 7 patients whose body weight was regularly measured and the mean weight gain

Table 1. Characteristics of the patients and therapeutic regimens.

Case number	Age (yr)	Sex	Liver cirrhosis	Type by image studies	Portal thrombus	Ascites	Multiple intrahepatic lesions	Extrahepatic metastasis	HBsAg	AFP (ng/ml)	Duration of disease before treatment	Previous therapies	Doses of recombinant interleukin-2 administered	Doses of adriamycin administered (on day 5)	Survival times (month)
1	15.5	M	–	Infiltrative	–	+	+	+, lung	+	23,485	2 weeks	Nil	30,000 U/kg/qBh/9 days	30 mg/m^2	6
2	43	M	+	Nodular	–	–	+	+, lung	+	>70,000	6 months	Transcatheter arterial embolization, systemic and intra-arterial chemotherapy	30,000 U/kg/qBh/4 days	–	9
3	43	M	+	Infiltrative	+	–	–	+, lung and lymph node	+	71,236	3 months	Nil	10,000 U/kg/qBh for 2 days, 20,000 U/kg/qBh for 2 days, 30,000 U/kg/qBh for 5 days	30 mg/m^2	7
4	46	M	+	Nodular	–	–	+	–	+	6,337	16 months	Right partial lobectomy, radiotherapy	30,000 U/kg/qBh/9 days	60 mg/m^2	18
5	50	M	+	Nodular	–	–	+	+, lung	+	696	8 months	Right lobectomy	30,000 U/kg/qBh/1 day	–	10
6	50	M	+	Infiltrative	+	+	+	–	+	66,136	7 months	Intra-arterial chemotherapy	30,000 U/kg/qBh/18 days	30 mg/m^2	9
7	62	F	+	Infiltrative	+	–	–	+, brain and bone	+	>134,000	11 months	Transcatheter arterial embolization	30,000 U/kg/qBh/4 days	–	12
8	63	M	–	Infiltrative	+	+	–	–	ND	4	4 months	Nil	26,000 U/kg/qBh/12 days	30 mg/m^2	8
9	68	M	+	Infiltrative	+	+	+	–	+	34	6 months	Intratumor ethanol injection	21,000 U/kg/qBh/3 days	30 mg/m^2 (on day 3)	9

ND: not done

Table 2. Adverse reactions to immunochemotherapy with recombinant interleukin–2 and adriamycin

Symptoms and signs	patient number	%
Fever, chills	9/9	100
Anorexia	9/9	100
Malaise	9/9	100
Nausea, vomiting	7/9	78
Diarrhea	7/9	78
Cough	6/9	67
Chest X–ray:		
Interstitial infiltration	4/9	44
Pleural effusion	5/9	56
Dyspnea	4/9	44
Edema	8/9	89
Weight gain	6/7	86
Abdominal distension	8/9	89
Enlarged liver	5/9	56
Oliguria	2/7	28
Hypotension	7/9	78
Skin rash	6/9	67
Mucositis	6/9	67

was 7.8%. Oliguria with daily urine output less than 500 ml was noted in 2 (28%) of 7 patients. Within one week after therapy, diuresis occurred, edema subsided, and body weight decreased promptly. Hypotension occurred in 7 patients (78%) with an average mean arterial pressure drop of 26.8 mmHg. Hypovolemic shock occurred in one patient and dopamine infusion was needed in 2 patients. Six patients (67%) had skin eruptions: one had facial erythema and the other 5 had generalized erythematous macular rashes. Generalized desquamation occurred in 2, and hyperpigmentation in one after fading of the rashes.

One patient (case 5) received only 2 doses of rIL-2 due to intolerable fever, chills, and hallucination, and therefore only 8 patients had adequate hemograms for analysis. All patients had normochromic, normocytic anemia, 7 (88%) had thrombocytopenia (platelet count $<100{,}000/mm^3$), and 7 (88%) had leukopenia (WBC count $<4{,}000/mm^3$). The nadir was seen at the third week of treatment. Lymphopenia (lymphocyte count $<1{,}500/mm^3$) was found in all patients but rebounding lymphocytosis was not seen. Neutropenia (absolute neutrophil count $<1{,}000/mm^3$) developed in 5 patients (71%) including one with transient complete absence of neutrophils. Eosinophilia (eosinophil count $<400/mm^3$) was observed in 5 patients (71%).

Six patients (75%) had elevated direct and indirect bilirubin levels. Serum transaminases (SGOT, SGPT) were increased moderately in 4 patients (50%), decreased in two, and fluctuated in the remaining two. Transient elevation of lactate dehydrogenase (LDH) and alkaline phosphatase (Alk-P) were encountered in 3 patients (38%). Serum albumin and globulin decreased in 5 patients (62%). Azotemia developed in 7 patients (88%). The mean BUN value increased from 14.1 mg/dl to 45.6 mg/dl and mean creatinine was greater than 10 mg/dl in all patients. Elevation of uric acid occurred in 6 patients (75%) with a mean increase of 6.4 mg/dl. Hyponatremia occurred in 7 patients (88%). Impairment of renal function usually recovered within 2 weeks after completion of therapy.

All of the adverse reactions usually appeared within several days after starting treatment and recovered within one week after discontinuation of IL-2 administration.

Immunological responses

Table 4 shows the changes of the numbers of NK cells, NK and LAK activities in the course of IL-2 treatment. The number of NK cells, NK and LAK activities increased from $386 \pm 88/mm^3$, $12.2 \pm 4.6\%$ and $6.8 \pm 1.9\%$ before treatment to $1{,}954 \pm 406/mm^3$, $48.8 \pm 15.3\%$ and $52.6 \pm 10.6\%$ during IL-2 infusion (6th day) and then decreased rapidly to $492 \pm 79/mm^3$, $35.4 \pm 10.2\%$ and $27.2 \pm 8.5\%$ one day after completion of IL-2 therapy.

DISCUSSION

Incubation of lymphocytes with IL-2 can generate lymphokine-activated killer (LAK) cells that possess non-specific cytotoxicity against a variety of tumors, [10,11,18] and remarkable immunotherapeutic effects of IL-2 and LAK cells have been demonstrated in murine models [11,18] and in humans. [19-21]

The activity and production of IL-2 were found to be reduced in chronic HBV infection. [22] Pilot studies of IL-2 in chronic hepatitis B had shown some benefit. [23,24] Decreased spontaneous natural killer (NK) activities and LAK activity were observed in HCC patients, and IL-2 could enhanced both NK and LAK activities in these patients, although to a lesser degree than those

of controls.[12,13,25,26] The decreased killer cell activities and IL-2 responsiveness were attibuted to an altered subpopulation ratio and a functional defect of NK effector cells.[25] In addition, a marked local lymphocytic infiltration in HCC specimens correlated with a significant better survival rates.[27] Our previous studies showed that LAK cells could be generated by incubating peripheral blood mononuclear cells of HCC patients with IL-2 and could lyse HCC cell lines but not normal hepatocytes.[12] Moreover, it had been demonstrated that impairment of immune cytolytic function caused by chemotherapeutic agents could be rescued with pretreatment of IL-2.[14,15] Thus, HCC probably is a candidate for IL-2 immunochemotherapy.

Nine cases of HCC in this study received various periods of high-dose rIL-2 and adriamycin; however, *no one showed objective response.* Both the hepatic tumors and pulmonary metastatic nodules continued to grow rapidly in eight patients. In our previous trial, rIL-2 and LAK cells were administered to 2 boys (seven years and nine years old, respectively) with end-stage primary HCC. After two successive courses of treatment, tumors still progressed rapidly and these 2 patients died 3 and 5 months, respectively, after onset of disease.[13] Failure of systemic administration of LAK and rIL-2 in the treatment of adult HCC patients was also reported recently.[28] Another clinical trial using LAK cells and IL-2 of much lower dosage administered through various routes also reported no clinical remission, although in some patients a significant decrease in serum alpha-fetoprotein level was observed.[29] However, by long-term administration of lower dose of IL-2 (15 μg/day for 14-64 days) and LAK cells (10^9-10^{10} cells/day) of 1-2 times per week, Onishi *et al*[30] were able to decrease the tumor size in 2 of 2 patients with

Table 3. Laboratory data after immunochemotherapy with recombinant interleukin–2 and adriamycin

Abnormalities	Patient number	%
Anemia	8/8	100
Thrombocytopenia	7/8	88
Leukopenia	7/8	88
Neutropenia	5/7	71
Lymphopenia	7/7	100
Lymphocytosis	0/7	0
Eosinophilia	5/7	71
Elevated bilirubin	6/8	75
Elevated SGOT, SGPT	4/8	50
Elevated LDH	3/8	38
Elevated Alk–P	3/8	38
Decreased albumin & globulin	5/8	62
Decreased cholesterol	8/8	100
Elevated triglyceride	5/8	62
Elevated creatinine	7/8	88
Elevated BUN	7/8	88
Elevated uric acid	6/8	75
Hyponatremia	7/8	88

Table 4. The changes of the number of NK[a] cells, NK and LAK[b] activities in the course of immunochemotherapy with recombinant interleukin–2 and adriamycin in seven patients with hepatocellular carcinoma

	NK cells (/mm^3)	NK activity (% lysis)	LAK activity (% lysis)
Before	386 ± 88*#	12.2 ± 4.6$	6.8 ± 1.9£
During	1,954 ± 406#	48.8 ± 15.3$	52.6 ± 10.6£
After	492 ± 79	35.4 ± 10.2	27.2 ± 8.5

a NK: Natural killer cell
b LAK: Lymphokine–activated killer cell
* Mean ± SD, #$£ $P < 0.001$.

solitary tumors and to prevent progressive increase of tumor size in seven patients. A decrease of more than 35% of serum alpha-fetoprotein was observed in 4 of 9 patients in that study. A transient decrease of alpha-fetoprotein level was also found in 6 of our patients. Since several crude cytokine preparations have been demonstrated to decrease alpha-fetoprotein synthesis by hepatoma cell lines,[31] the decrease of alpha-fetoprotein during rIL-2 therapy most probably results from a direct or indirect effect of rIL-2 on hepatoma cells or hepatocytes, not necessarily reflecting the cytolysis of tumor cells themselves.

In this study, a wide variety of severe adverse reactions related to IL-2 adminsitration was encountered and they were similar to those reported previously.[19,32,33] The greater incidence and severity of thrombocytopenia and neutropenia found in this study, as compared to those reported by Ettinghausen *et al,*[34] might result from simultaneous use of adriamycin in this study.

Although the present study (Table 4) and our previous reports have repeatedly shown that IL-2 is capable of increasing NK cell numbers, augmenting NK activity and generating LAK activity both *in vitro*[12] and *in vivo,*[9,13] IL-2 in combination with adriamycin failed to treat HCC as the survival time of patients in this study was not prolonged when compared to that of HCC patients treated with other methods. This is due mainly to the far advanced stage of the tumors. However, in view of the capability of LAK to kill autologous or allogeneic fresh hepatoma cells or cell lines *in vitro,*[12] it may be still worth trying to administer IL-2 directly into the tumor tissue in combination with other standard methods, especially in the early stages of hepatocellular carcinoma, as described in other cancers.[35-37]

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