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Treatment of hepatocellular carcinoma utilizing lymphokine-activated killer cells and interleukin-2

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Summary. This paper is a report on adoptive immunotherapy involving consecutive injections of recombinant interleukin-2 and lymphokine-activated killer (LAK) cells in the treatment of hepatocellular carcinoma. Peripheral blood lymphocytes, obtained by leukopheresis, acted as the activated killer cells with a co culture of recombinant interleukin-2 in the culture system. After 4 days, the activated killer cells were returned into the patients' bodies intra-arterially and intravenously. No complete remissions or partial remissions have resulted, although five of the seven patients showed a significant decrease in their serum α-fetoprotein levels after treatment. In addition, one case showed a patent portal truncus while another indicated the appearance of central necrosis on the computerized tomograph scan. Although the period of observation was short, there were no recurrences after the combination therapy of tumor resection and LAK adoptive immunotherapy. It might be difficult to treat hepatocellular carcinoma with adoptive immunotherapy alone, but there is some possibility of conducting therapy for hepatocellular carcinoma after removing the majority of the tumor cells by surgical resection and transcatheter arterial embolization therapy. This conclusion indicates, at least theoretically, that adoptive immunotherapy will be suitable in the treatment of hepatocellular carcinoma as one of the combination therapies with the two major forms of treatment mentioned above.

Introduction

Although various therapeutic efforts have recently been made for the treatment of hepatocellular carcinoma, such as hepatic artery ligation, temporary or consecutive hepatic arterial infusion of anticancer agents and transcatheter arterial embolization, the mortality rate has not decreased dramatically compared to that from other malignant tumors in humans. Surgical resection might therefore prove to be of greatest use in the treatment of these patients. However, there are many restrictions on surgical resection where the tumor is localized in one lobe with no evidence of intrahepatic and/or extrahepatic metastasis, and because the liver functions have to be preserved indefinitely. Partial palliation results temporarily, but the recurrence of tumors is frequent, until the patient succumbs. Thus, a more advanced treatment of hepatocellular carcinoma is needed. Recently not only active immunotherapy but also passive immunotherapy [12] has been used in the treatment of malignant tumors in humans. Adoptive immunotherapy utilizes lymphokine-activated killer (LAK) cells [14, 15, 16, 19] induced by interleukin-2 [10], one of the significant cytokines which can enhance both natural killer cells [4] and LAK cell activity [3]; these non-specific killer cells have the potential for actively killing tumor cells [7, 8, 9, 14]. In the current study we used LAK adoptive immunotherapy in the treatment of human hepatocellular carcinoma with various administrations of LAK cells and interleukin-2.

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Materials and methods

Patients. The patients studied had varying performance scores on the Eastern Cooperative Oncology Group scale. Three patients had received no previous treatment. Four patients with marked portal thrombosis had previously received a one-shot injection of adriamycin via a hepatic arterial catheter. Two of them had had surgical resection. Transcatheter arterial embolization therapy had been previously used on one patient. The background of all the patients is shown in Table 1.

Administration procedure. All the patients were treated with recombinant interleukin-2-activated cells (LAK cells). Six patients were treated with LAK cells by intra-arterial infusion with the arterial catheter inserted from the femoral artery. One patient was treated with LAK cells directly via a hepatic arterial cannulated tube. Three of the patients were administered LAK cells intravenously. Five patients received intramuscular injections of interleukin-2 while the others received interleukin-2 via an intra-arterial catheter both directly and constantly.

Lymphokine-activated killer cell preparation. Peripheral mononuclear blood cells were obtained by leukopheresis (CS3000) from the patients with hepatocellular carcinoma. These cells were then prepared using the Ficoll-Conray gradient technique. These cells were washed in a phosphate-buffered saline solution, and were suspended at 2×10^6 cells/ml in RPMI 1640 supplemented by 2% autologous human serum or 2% allogenic human AB serum. The cell suspension with 4 U/ml interleukin-2 was poured into

Table 1. Background of all patients

Case no.	Age (years)	Sex	PS[a]	Image finding	Intrahepatic metastasis	Portal thrombus	Serum tumor marker
1	56	F	0	Nodular[b] (E2)[c]	Yes	Yes	AFP[d]
2	54	F	0	Nodular (E1)	Yes	No	AFP
3	76	M	1	Nodular (E1)	No	No	AFP
4	50	M	0	Nodular (E1)	No	Yes	(−)
5	51	M	0	Nodular (E1)	No	No	(−)
6	54	M	2	Diffuse (E4)	Yes	Yes	AFP
7	59	M	0	Massive (E2)	Yes	Yes	AFP
8	62	F	0	Nodular (E1)	No	No	AFP
9	39	M	1	Diffuse (E3)	Yes	Yes	PIVKA II[e] [5]
10	62	M	1	Massive (E2)	Yes	Yes	AFP

[a] Eastern Cooperative Oncology Group scale
[b] Classification by Eggels
[c] The general rules of the clinical and pathological study of primary liver cancer by liver cancer study group of Japan: E1, less than 20%; E2, 20–40%; E3, 40–60%; E4, more than 60% (tumor-bearing area in the liver)
[d] AFP, α-fetoprotein
[e] PIVKA II, prothrombin induced vitamin K antagonist II

plastic culture bottles and incubated for 4–5 days in 5% CO_2 at 37° C. The cells were then harvested, washed three times in the phosphate-buffered saline solution and resuspended in saline. These LAK cells were used in adoptive immunotherapy with this protocol.

Results

Administration of LAK cells and interleukin-2 (Table 2)

The average dose of interleukin-2 was 1000–2000 U/day for each patient. Regardless of the dosage, interleukin-2 was continuously infused until the side-effects precluded further administration. The maximum dose was 149000 U via an intrahepatic arterial cannulated tube for approximately 5 months. During the course of treatment, half of the patients received interleukin-2 through the intrahepatic artery with the tube inserted into the femoral artery using the Seldinger method. The other half received intramuscular injections of interleukin-2 twice a day.

Fundamentally a one-week cycle of leukopheresis and LAK cell reinfusion was given. However, these intervals were subject to change because of the various side-effects. The maximum dose was 17.1×10^9 cells for the patients of case 1. The average number of LAK cells was 2.1×10^9 each time. Six patients received LAK cells directly through an intrahepatic artery injection. Two of the other patients were injected with the LAK cells intravenously, while the other two were injected intravenously and intra-arterially, respectively.

Response to treatment (Table 3)

Among the ten patients treated with LAK cells and interleukin-2, neither complete nor partial remission was detected during the course of this treatment. However, four patients showed a clinical response after treatment. Furthermore, the serum level of α-fetoprotein decreased dramatically after treatment in six patients and the serum level of PIVKA II(5) also decreased in another case. The mean reduction ratio of the serum α-fetoprotein levels was 56.2%.

Three patients had no previous treatment and showed a marked reduction in their serum α-fetoprotein levels. Two of them had received treatment after surgical resection of the tumor. One of the two patients had showed a portal invasion with tumor thrombosis histologically. Dur-

Table 2. Procedure of adoptive immunotherapy

Case no.	Previous therapy[a]	Total dose of IL-2[b] (kU)	(via)[c]	$10^9 \times$ Total dose of LAK cells	(times)	(via)
1	Non	149	(i.h.a.)	17.1	(10)	(i.h.a.)
2	Non	36	(i.h.a.)	7.2	(4)	(i.h.a.)
3	Non	53.5	(i.v.)	16.0	(5)	(i.v.)
4	Tumor resection	45	(i.h.a.)	6.6	(3)	(i.h.a.)
5	Tumor resection	25	(i.h.a.)	7.8	(4)	(i.h.a.)
6	TAE	42	(i.h.a.)	3.4	(3)	(i.h.a.)
7	ADR one shot	24	(i.m.)	4.6	(5)	(i.v., i.h.a.)
8	ADR one shot	30.5	(i.m.)	9.0	(2)	(i.h.a.)
9	ADR one shot	455.5	(i.m.)	17.0	(8)	(i.v., i.h.a.)
10	ADR one shot	40	(i.m.)	12.0	(5)	(i.v.)

[a] TAE, transcatheter arterial embolization therapy; ADR, adriamycin
[b] IL-2, interleukin-2
[c] i.h.a., intrahepatic artery; i.v., intravenous; i.m., intramuscular

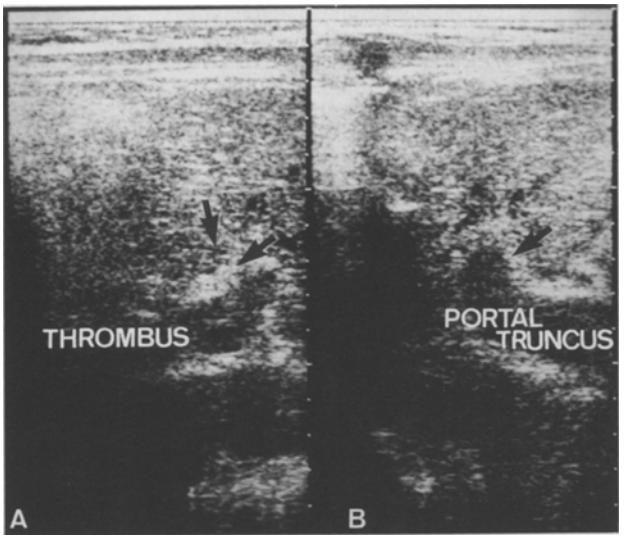

Fig. 1. The ultrasonographic findings of case 9. Before treatment, a portal thrombus in the main truncus was observed **A**, while after treatment a patent portal truncus was found in the same area **B**

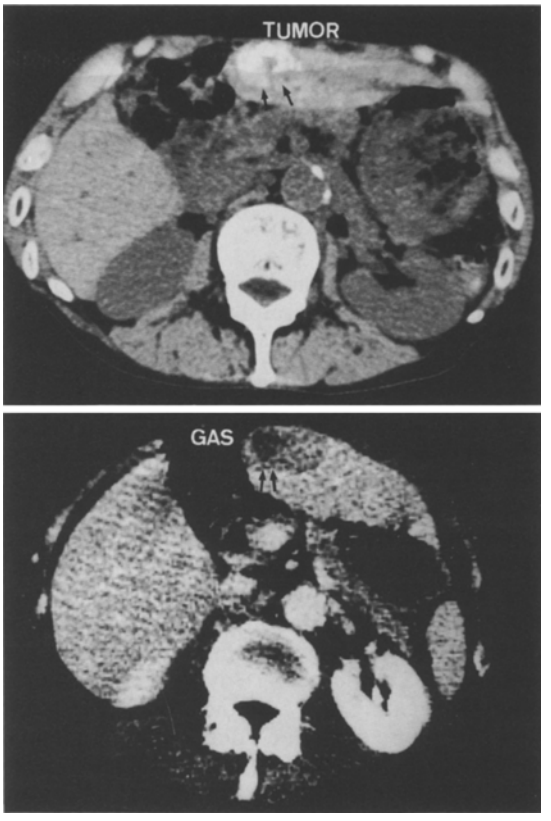

Fig. 2. The computerized tomography scan of case 1. *Top:* on the left lobe, a small tumor nodule could be detected after lipiodolization. *Bottom:* after LAK adoptive immunotherapy, gas formation was observed at the center of the tumor, corresponding well with the serum levels of diminished α-fetoprotein

ing the 8 months of observation, there were no signs of a recurrence in either case. One of them had received treatment after transcatheter arterial embolization therapy, and a 14.3% reduction of the serum α-fetoprotein levels was obtained. Four of the patients had received treatment after intra-arterial one-shot injections of adriamycin, and two of the four showed a marked decrease of the serum tumor markers.

One of the six patients who had had portal thrombosis showed a dramatic patent portal truncus after treatment (Fig. 1). Another case showed increased central necrosis, which was indicated as a gas formation on the computerized tomography scan and in ultrasonography (Fig. 2).

Side-effects (Table 3)

The LAK cells were incubated in a culture medium for 4 days. In order to rule out the possibility of bacterial contamination, these cells were examined in a bacterial and viral test, and found to be clear. Fever and hypotension occurred in all the patients during treatment. These anticipated side-effects were lessened through the use of acetaminophen, indomethacin and dopamine. One case resulted in mild renal failure after treatment such as oliguria, and the excretion of sodium decreased temporarily. DIC and severe shock occurred in one patient (case 2). Although a

Table 3. Results of the treatment

Case no.	CR[a]	PR	NC	PD[a]	PS[a]	Tumor marker[b] (ng/ml)		Remarks	Side-effect
1			0		0–0	AFP	32800–8000		Fever, hypotension
2			0		0–0	AFP	907–44		Shock, DIC[f]
3			0		1–0	AFP	412–157	[c]	Fever, hypotension
4			0		0–0	(–)			Fever, hypotension
5			0		0–0	(–)			Non
6			0		2–1	AFP	35–5		Renal failure
7			0		0–0	AFP	214–135		Fever, hypotension
8				0	0–1	AFP	121–118	[d]	Fever, hypotension
9			0		1–0	PIVKA	1270–492	[e]	Fever, hypotension
10			0		1–0	AFP	145–219		Fever, hypotension

[a] CR, complete response; PR, partial response; NC, no change; PD, progressive disease; PS, performance status
[b] AFP, α-fetoprotein; PIVKA II, prothrombin induced vitamin K antagonist II
[c] Gas formation can be observed at follow up computerized tomograph scanning at the center of tumor
[d] Progress
[e] Patent portal truncus after treatment
[f] DIC, disseminated intravascular coagulation

marked decrease in the level of serum α-fetoprotein was found, the patient suffered from sudden shock and DIC after the fourth time of LAK cell reinfusion (total 7.2×10^9 cells) and the continuous infusion of interleukin-2 (total 32000 U). After treatment, the patient was able to recover from the shock and DIC, but the serum level of α-fetoprotein gradually increased 3 months after the first treatment.

Discussion

Interleukin-2, which can act as an intracellular transmitter, has a varied biological and immunological potential. One of the major functions of interleukin-2 in humans is enhancing natural killer cells [4] and lymphokine-activated killer cell activity [3], as well as augmenting the generation of cytotoxic T-lymphocytes against autologous tumor cells [6, 18]. The production of recombinant interleukin-2 from *Escherichia coli* has been recently developed [13, 17]. Consequently, passive immunotherapy in the treatment of malignant tumors in humans and animals, utilizing interleukin-2, is beginning to be reported by several authors [11, 15, 19]. Rosenberg and his colleagues have reported the efficacy of LAK adoptive immunotherapy in the treatment of 106 cases with various malignant tumors. They stressed the significance of a 33% remission. However, this therapy was not used in the treatment of hepatocellular carcinoma in humans. Hitherto, one report on adoptive immunotherapy for the treatment of hepatocellular carcinoma has been published. Okuno et al. reported on the treatment of non-resectable hepatoma utilizing LAK cells generated from autologous spleen cells [11]. They observed a transient fall of the serum α-fetoprotein levels and a reduction of ascites fluid. Our current study has revealed clinical efficacy and a marked decrease of serum α-fetoprotein levels, although neither complete nor partial remissions occurred. In addition, some cases showed marked morphological changes on the computerized tomography scan, such as a patent portal truncus and gas formation. In principle, approximately 2.1×10^9 LAK cells could be returned to the patient each time, but the number of tumor cells was more than 100–1000 times the number of LAK cells. We therefore need a greater number of LAK cells and a more efficient administration procedure, in the form of a direct injection and/or direct infusion into the tumor tissues. For this purpose, effective LAK adoptive immunotherapy would be suitable after removing the majority of the tumor cells by surgical resection and transcatheter arterial embolization therapy. In addition interleukin-2 is necessary for the LAK cells to maintain their activity [9], so the continuous infusion of interleukin-2 should be done locally because of the rapid secretion from the kidneys [1, 2] and the existence of an interleukin-2 inhibitor [1, 7] in their sera. In conclusion, although LAK adoptive immunotherapy might not be the absolute therapy for the treatment of hepatocellular carcinoma, we could at least reveal the efficacy of this therapy clinically and morphologically. The combination therapy of LAK adoptive immunotherapy and both surgical resection and transcatheter arterial embolization therapy in the treatment of hepatocellular carcinoma might bear fruit in the near future.

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