Effect of Lymphokine-activated Killer Cells with or without Radiation Therapy against Malignant Brain Tumors

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Abstract

The use of autologous lymphokine-activated killer (LAK) cells to treat malignant brain tumors was evaluated in 10 patients, one with metastatic malignant melanoma and nine with malignant glioma. LAK cells were obtained by culturing autologous peripheral blood lymphocytes with human recombinant interleukin-2 (rIL-2) for 7–28 days. All patients underwent surgery to remove as much tumor as possible and an Ommaya reservoir was implanted in the tumor cavity. Two of the 10 patients had received radiotherapy elsewhere, so were treated with LAK cells alone. Eight patients were treated with a combination of LAK cells and radiotherapy, using 1.8–2.0 Gy fractions given five times a week with a total dosage between 54 and 65 Gy. LAK cells and rIL-2 were injected to the tumor cavity *via* the Ommaya reservoir once a week for inpatients and once a month for outpatients. The duration of the LAK therapy ranged from 3 to 23 months (mean 13.7 mos). Neuroimaging evaluation revealed two complete responses, three partial responses, four no changes, and one progressive disease. In one patient with pontine glioma, the Karnofsky performance score was raised from 20 to 60. There were no side effects after the injection of LAK cells and rIL-2. The results suggest low-dose LAK therapy is a useful and safe treatment modality for malignant brain tumors.

Key words: interleukin-2, lymphokine-activated killer cells, malignant brain tumor, radiotherapy

Introduction

Surgical therapy, radiotherapy, and chemotherapy are used to treat malignant brain tumors, but are limited by the associated physical and chemical damage to the normal tissues. Immunological treatments are under intensive research because they are tumor-specific and harmless to normal tissue. Lymphokine-activated killer (LAK) cells are a potential agent obtained by culturing lymphocytes with interleukin-2 (IL-2).⁶⁾ However, the clinical results are controversial, with some workers concluding that immunotherapy with LAK cells would be ineffective for malignant brain tumor,^{3,17)} while others have suggested the method is efficient and safe for patients with brain tumor.^{11,18)} In these studies, no cases of cure have been presented and the ratio of cases with

remission is relatively low.

LAK cells can also be induced from the peripheral blood lymphocytes (PBL) of the tumor-bearing host, and the activity persists in patients with glioma⁴⁾ and healthy subjects. LAK cells are widely accepted to be broad-spectrum, easily induced, and harmless to normal tissues. Many clinical trials have evaluated the use of autologous LAK cells against malignant tumors.^{3,10,11,17,18)} Recently, several trials have attempted to improve the activity of LAK cells using the specific targeting technique or plastic adherent LAK cells. The specific targeting technique involves introducing two antibodies, the anti-CD3 and anti-glioma monoclonal antibodies, into the LAK cell to intensify the binding activity to the target tumor cell.¹⁵⁾ The preliminary results suggest a longer survival time for the patients than in normal LAK therapy. A culture supernatant of plastic adherent LAK cells can also enhance the lytic activity of LAK cells in the

Table 1 Patient data

Case No.	Age/Sex	Histology	Location	Previous treatment	Tumor removal	KPS
1	47/M	malignant melanoma	lt F-T	—	partial	60
2	41/M	astrocytoma III	lt P	—	partial	90
3	63/F	glioblastoma multiforme	lt F-T	Op + RT	partial	30
4	29/M	astrocytoma III	rt P	—	partial	60
5	40/F	astrocytoma III	rt F	Op	—	90
6	25/F	astrocytoma III	pons	Op + RT + chemo	—	20
7	67/F	astrocytoma III	rt F-T	chemo	partial	90
8	70/F	astrocytoma III	lt F-T	—	partial	90
9	38/F	astrocytoma III	rt F	—	subtotal	90
10	72/F	astrocytoma III	rt F-T	—	partial	40

chemo: chemotherapy, F: frontal lobe, KPS: Karnofsky performance score, Op: surgery, P: parietal lobe, RT: radiation therapy, T: temporal lobe.

glioma system of rats.¹⁶⁾

The cytotoxic mechanism of LAK cells is thought to be recognition of the antigenic determinants of tumor cells⁹⁾ followed by production of cytotoxic factors.⁷⁾ Our previous animal experiments revealed that 5 Gy of irradiation enhanced the natural killer (NK) or LAK cell activity,^{13,14)} due to an increase in the release of cytotoxic factors from LAK cells or NK cells following irradiation.

In this study, 10 patients with malignant brain tumors were treated with a combination of LAK cells and irradiation, or LAK cells alone, and evaluated by computed tomography (CT) or magnetic resonance (MR) imaging, and the neurological status including the Karnofsky performance score.

Materials and Methods

Ten patients, one with metastatic malignant melanoma and nine with malignant astrocytoma, were treated with a combination of LAK cells and irradiation (8 patients) or LAK cells alone (2 patients) (Table 1). Informed consent was obtained from all patients or guardians.

PBL were isolated from 100 ml of heparinized blood from each patient by a standard Ficoll-Conray density-gradient centrifugation technique. After centrifugation, the red blood cells were returned to the patient. PBL were suspended in RPMI 1640 (Nissui Co., Tokyo) at 1×10^6 /ml supplemented with 1–2% human AB serum and 1000 U/ml recombinant human IL-2 (rIL-2) (Shionogi Co., Ltd., Osaka) and incubated for 7–28 days. $1-10 \times 10^7$ LAK cells were resuspended in 0.5 ml of Hanks' balanced salt solution (HBSS) (Whittaker Bioproduct, Walkersville, Md., U.S.A.) supplemented with 1×10^4 U/ml of rIL-2.

The tumor was treated surgically in eight patients. A catheter was placed in the postoperative cavity connected with an Ommaya reservoir (Baxter Healthcare Co., Deerfield, Ill., U.S.A.) placed beneath the galea. Only one patient (Case 5) received surgery to implant the Ommaya reservoir without tumor removal because the recurrent tumor had seeded to the ventricular system. Case 6 was not treated because she had received surgery before. Conventional ionizing radiotherapy (1.8–2 Gy per fraction, five times a week) was given to eight patients, with total radiation doses ranging from 54 to 65 Gy. Eight of the 10 patients received a local injection of LAK cells and rIL-2 via the Ommaya reservoir once a week during radiotherapy. The other two patients (Cases 3 and 6) were treated with LAK cells alone via the Ommaya reservoir because they had previously received irradiation at another hospital.

Injection of LAK cells was continued once a week during hospitalization and once a month for outpatients. The duration of LAK therapy ranged from 3 to 23 months (mean 13.7 mos). Injections of LAK cells consisted of 0.5 ml HBSS suspension injected into the Ommaya reservoir with a 26-G needle and tuberculin syringe.

Patient response was usually evaluated at the end of radiotherapy by CT or MR imaging and neurological examination. However, the two patients who received LAK therapy alone were evaluated during the therapy. CT or MR imaging responses were graded as complete response (CR): disappearance of the tumor for at least 4 weeks, partial response (PR): a greater than 50% reduction in tumor size continuing for more than 1 week, no change (NC), and progressive disease (PD). The tumor size was measured using the greatest width and length. The neurological status was evaluated as

Table 2 LAK therapy and evaluation

Case No.	LAK therapy		Radiation dose (Gy)	Evaluation		Outcome (survival time)
	No. of injections	Total cell No. ($\times 10^7$)		CT and MR imaging	KPS	
1	37	250	60	PR	60	died (16 mos)
2	39	370	61	PR	90	died (32 mos)
3	20	73	—	NC	30	died (22 mos)
4	15	65	54	PD	60	died (10 mos)
5	31	90	60	CR	90	alive (90 mos)
6	23	87	—	PR	60	alive (24 mos)
7	17	44	65	NC	90	died (17 mos)
8	15	65	64.8	CR	90	alive (24 mos)
9	23	87	64.8	NC	90	died (17 mos)
10	8	31	60	NC	40	alive (12 mos)

improved, no change, and worse simultaneously with the CT or MR imaging evaluation. Figure 1 shows the treatment and evaluation scheme.

Results

LAK cells were administered to 10 patients on more than 200 occasions. No complications including headache, high fever, convulsion, infection, or bone marrow suppression were detectable in any patient. CT or MR imaging evaluation revealed that two of the 10 patients showed CR after the radioimmunotherapy, three showed PR, four showed NC, and one showed PD (Table 2). The two patients (Cases 5 and 8) who showed CR both had astrocytoma grade III. Case 5 underwent subtotal removal of an astrocytoma grade II 4 years previously, and was readmitted because of multiple intraventricular tumors (Fig. 2), the diagnosis was based on neuroradiological findings only. Of the three patients with PR, one had a metastatic malignant melanoma

(Case 1, Fig. 3) and two had astrocytoma grade III (Cases 2 and 6). The four patients with NC had astrocytoma grade III or glioblastoma multiforme. Case 4 showed ventricular seeding of astrocytoma grade III following the combined radiation and LAK therapy and was evaluated as PD.

Neurological improvement was detected in one patient with pontine glioma (Case 6, Fig. 4). The level of consciousness improved with a marked decrease in intracranial pressure. The Karnofsky performance score of this patient reached 60 after 10 injections of LAK cells compared to 20 before starting the therapy. This patient was discharged 4 months after the initiation of LAK therapy. Other patients showed no neurological changes after the treatment. One patient (Case 1) contracted hydrocephalus, and subsequently received a ventriculoperitoneal shunt.

Discussion

The combined use of LAK therapy and irradiation to

Fig. 1 Treatment and evaluation schedule.

Fig. 2 Case 5. Postcontrast CT scans, demonstrating multiple enhanced masses in the third ventricle (arrow), lateral ventricle, and right high frontal lobe before treatment (upper), which disappeared 3 months after irradiation and LAK therapy (lower). This patient was evaluated as CR continuing for 24 months.

treat patients with malignant brain tumors achieved CR in two patients and PR in three. These results are more promising than in other studies using LAK cells, possibly because irradiation was used simultaneously with LAK therapy, and LAK therapy was continued for a relatively long period.

LAK cells were administered once a week during radiotherapy, as 95% of LAK cells injected into the tumor site survive for over 48 hours.¹⁰⁾ LAK cells injected into a surgical cavity combined with 4–6 Gy of irradiation per week should achieve enhanced cytotoxicity based on our previous experimental results.^{13,14)} NK cells are more radioresistant¹¹⁾ and retain their lytic activity after 10-Gy irradiation.⁸⁾ Our experimental data suggested that cytotoxic T cells did not lose their cytotoxic activity after 7.5-Gy irradiation (data not shown). Therefore, the lytic function of LAK cells should not be affected by 4- to 6-Gy irradiation.

Complications associated with administration of LAK cells seem to depend on the dose of IL-2 and the number of LAK cells. Previous trials have treated tumors using a large number of LAK cells.¹²⁾ Barba *et al.*³⁾ reported cerebral edema and neurological deterioration in all patients using a relatively high dose of IL-2 and a much larger number of LAK cells administered locally. Merchant *et al.*¹¹⁾ reported headaches, low-grade fevers, and lethargy in all their patients with a high dose of IL-2 (10^6 U) and local administration. Atkins *et al.*²⁾ reported hypothyroidism in 21% of patients treated by intravenous administration of IL-2 and LAK cells. They suggested that the hypothyroidism might be caused by IL-2 and LAK cells. In contrast, our study found no side effects. The low incidence of side effect might be due to injection of LAK cells and IL-2 into the local tumor cavity, the relatively small number of LAK cells used in each injection, and the low dose

Fig. 3 Case 1. Postcontrast CT scans, showing metastatic malignant melanoma evaluated as PR 9 months after surgery. A: preoperative, B: 3 weeks postoperative, C: 3 months postoperative, D: 9 months postoperative.

of IL-2 (1×10^4 U). Our results suggest that a small number of LAK cells and a low dose of IL-2 are adequate without causing complications.

The outcome of LAK therapy is very difficult to predict. Our study included four patients without change (NC) and one with PD in addition to the five patients in whom the therapy was effective (CR and PR). The reasons for the different responses are still unknown. The number and frequency of LAK cell administration were the same for the two groups. The response may depend on the induction of suppressor cells among LAK cells.

No study has compared the *in vitro* LAK cell activity and the clinical effect. However, Merchant *et al.*¹¹⁾ tested LAK cell activity using a NK-resistant Daudi cell line and evaluated the clinical course of these patients. The results seemed to show no significant correlation between clinical response and non-response. Our study included three astrocytoma grade III patients with relatively short survival times (Cases 4, 7, and 9). In Case 4, the tumor spread to the ventricular system from the beginning of treatment although the primary tumor appeared to shrink. Case 9 also showed ventricular seeding 4 months after the completion

of treatment. In this patient, the retinoblastoma (RB) gene was thought to be rearranged or point mutated (data not shown). The RB gene is thought to be one of the tumor suppressor genes.⁵⁾ Further investigation of tumor suppressor genes or oncogenes may allow prediction of tumor growth or the mechanism of tumor progression.

Low-dose LAK therapy, with or without radiation therapy, is potentially an effective and safe procedure for the treatment of patients with malignant brain tumor, but further clinical trials are necessary to obtain significant data.

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Fig. 4 Case 6. Serial T₁-weighted MR images with gadolinium-diethylenetriaminepenta-acetic acid, showing marked regrowth of pontine glioma despite radiotherapy and chemotherapy 3 months after surgery. Administration of LAK cells and rIL-2 was started 5 months after the surgery. The huge pontine glioma gradually decreased in size and was evaluated as PR. A: preoperative, B: postoperative, C: 3 months postoperative, D: 5 months postoperative, E: 11 months postoperative, F: 15 months postoperative.

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