

Observation of the Effects of *Lycium Barbarum* Polysaccharides (LBP) In Combination with LAK/IL-2 Therapy In the Treatment of 75 Cancer Patients

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Abstract: Seventy-nine advanced cancer patients in a clinical trial were treated with *Lycium Barbarum* polysaccharides (LBP) in combination with Lymphokine Activated Killer cell (LAK) and Interleukin-2 (IL-2). Initial results of the treatment indicated that regression of cancer was achieved in 75 of the 79 patients with various cancers. Cancers treated in this trial included malignant melanoma, renal cell carcinoma, colorectal carcinoma, lung cancer, nasopharyngeal carcinoma, and malignant hydrothorax. The response rate of patients treated with the LBP/LAK/IL-2 combination was 40.9% while that of patients treated with LAK/IL-2 only was 16.1% ($P < 0.05$). In addition, the mean length of time patients treated with the LBP/LAC/IL-2 combination therapy remained in remission was significantly greater than for patients in the LAK/IL-2 only treatment group. The LBP combination treatment led to a considerable increase in Natural Killer (NK) and LAK cell activity. These results indicate that LBP can be used as an adjuvant in the biotherapy of cancer.

LAK/IL-2 therapy has proven to be effective in treating advanced cancer patients, particularly in patients who respond poorly to radiation therapy. However, LAK/IL-2 therapy has not been widely used clinically because of patient intolerance of side effects. Historically, advanced melanoma has shown a sensitivity of approximately 40% to LAK/IL-2 therapy. We hoped the addition of *Lycium barbarum* to the LAK/IL-2 therapy would enhance the therapeutic effect on some of the more difficult cancers to treat, as well as lessen drug side effects.

In vivo studies of LBP from the Chinese herb, wolfberry, have reported enhanced LAK/IL-2 activity in the elderly. Based on previous reports, we conducted a clinic trial to combine LAK/IL-2 and LBP to treat advanced cancer patients from February 1992 to November 1993.

Materials and Methods

1. Subjects: all patients were diagnosed with cancer by both clinical and pathological confirmation. They had been treated with chemotherapy, radiation therapy, or surgery, and have not seen satisfactory results. All anti-cancer treatment had ceased one month or more before entering our clinical trial. Patients with severe heart, lung, liver or kidney dysfunction, allergies to biotherapy, and pregnant or lactating women were excluded. Four cases, which dropped out the study before November 1, 1993, were not included in this report.

2. Equipment and drugs: a) Automatic blood cell suspensor CS-3000 (Baxter), b) Electrophoresis grade of recombinant IL-2 (Cetus), c) LBP (Military Academy of Medical Science No. 6 Institute), d) 1 liter semi-permeable cell culture bag PL 732 (Baxter), e) Type AB serum (Shanghai Blood Bank), 6) CO₂ incubator and isotope.
3. LAK cell preparation: The mononuclear cells in peripheral blood (PBMNC) were separated and collected by CS-3000 as usual. Almost all fibrins and most erythrocytes were removed. The remaining cells were then adjusted in the culture media PRMI-1640 at the concentration of 1×10^6 cells per ml. IL-2 was added to the cultures with the final concentration at 1000 IU per ml. These cells were incubated at 37°C and 5% CO₂ with 100% humidity for 4-6 days. Culture media solution was replaced with fresh culture every 48-72 hours. The number of LAK cells were calculated, and their anti-tumor activity and contamination were inspected before collecting. The LAK (10^{10} - 10^{11}) cells were then washed and added to a 5% gluconate solution containing 1% albumin and 2×10^5 u of IL-2 and ready for IV application.
4. Treatment: Patients were randomly divided into 3 groups (I, II, and III). Group I took an oral dose of LBP 1.7mg/kg per day for four weeks prior to LAK/IL-2 treatment and continued taking LBP until the end of the study. Group II was given a placebo prior to LAK/IL-2 treatment. Group III was given LBP alone (not included in the report due to an insufficient number of patients).
 - a) Systemic Adoptive Immunotherapy:

Beginning week 5, IL-2 was injected subcutaneously at 2.0×10^4 u/ml per day and intravenously at 1.0×10^4 u/ml per day in addition to LBP or placebo for four days in Group I and II. On day five, peripheral blood mononuclear cells (PBMNCs) were obtained from patients and incubated for two days for induction into LAK cells. These LAK cells were then infused into the patients at 1.3×10^{10} per day along with IL-2 at 2×10^4 u/ml per day for three days (day 7-9).
 - b) Thoracic immunotherapy:

After closed thoracic drainage, IL-2 was administered by intra-thoracic injection at 1.0×10^5 per day to patients with malignant hydrothorax for seven days. On day 10, PBMNCs were obtained from hydrothorax and incubated *in vitro* for 2 days for inducing into LAK cells. These LAK cells were then administered through intra-thoracic injection at 1.9×10^{10} u/ml per day along with IL-2 at 2×10^4 u/ml per day for three days (days 13-15).
5. NK and LAK activity measurement: In house Lab protocol was followed and K562 And /Daudi were used as target cells.
 - i. Effectiveness (WHO standard): Complete remission (CR): tumor disappeared over 4 weeks. 2) Partial remission (PR): the total maximum diameter of the primary and the metastatic tumor reduced over 50% for at least 4 weeks. 3) Medium remission (MR): the total maximum diameters

of tumors reduced 25% to 50%. 4) Stable (SD): monthly growth rate of the tumor was less than 25% and no new growth found. 5) Progressive (PD): the monthly growth rate of tumors was 25% and metastatic tumors were found.

$$\text{Effectiveness} = \frac{\text{CR} + \text{PR}}{\text{Total n.}} \times 100\%$$

- ii. Statistic analysis: t test and X^2

Results:

- Treatment overview and evaluation:* The study coordinated 5 hospitals. Table 1 and 2 summarize the treatment of 75 patients through November 1, 1993. The effective rate found in the LBP/LAK/IL-2 combination group was 40.9% (18 in 44 cases). The effective rate in the LAK/IL-2 only group was 16.1% (5 in 31 cases). Significant differences were seen in treatment results between the two groups. This clinic trial treated various cancers. The LBP/LAK/IL-2 combination therapy showed effectiveness in treating all melanoma, renal carcinoma, colorectal carcinoma, lung cancer, nasopharyngeal carcinoma and malignant hydrothorax cases. The effectiveness rate was higher and lasted longer in the LBP/LAK/IL-2 combination group than in the LAK/IL-2 only group. The side effects associated with the therapy in both groups were similar, including: fever (90%), chills (45%), discomfort (60%), and weight gain (10%).
- Activity of NK and LAK before and after the treatment:* The NK activity in PBMNC and LAK activity induced by IL-2 500u/ml (3 days, titer 80:1) changed significantly after the treatment with $P < 0.05$ and $P < 0.001$ (Table3) respectively.

Table 1: Treatment Summary

Hospitals	Total case	LBP/LAK/IL-2		LAK/IL-2	
		Cases treated	Effective case	Case treated	Effective cases
Shanghai Changhai Hospital	34	16	6	18	3
Shanghai Jinshan Hospital	15	8	3	7	1
Hudong Hospital	9	9	4	-	-
Shanghai 85 th Hospital	11	5	3	6	1
Shanghai Chongming Hospital	6	6	2	-	-
Total	75	44	18 (40.9%)	31	5 (16.1)

Table 2: Various Cancers Treated

Cancer cases		LBP/LAK/IL-2					LBP/LAK/IL-2				
		Case	CR	PR	MR	Period ($\bar{x} \pm s$ month)	Case	Effective Case C R	PR	MR	Period ($\bar{x} \pm s$ month)
Renal Cancer	6	3	1	1	2	4.5±1.4	1	0	1	0	3.8
Melanoma	5	4	1	2	1	3.5±3.7	1	0	1	0	3.2
Colorectal Carcinoma	26	17	0	6	1	5.3±4.2	9	0	1	3	4.5±3.2
Lung Cancer	21	11	0	4	1	3.9±3.8	10	0	1	2	3.7±2.8
Liver Carcinoma	5	2	0	0	1	1.8	3	0	0	1	1.4
Nasopharyngeal Carcinoma	2	2	0	0	1	2.4	0	0	0	0	0
Breast Cancer	4	2	0	0	0	0	2	0	0	0	0
Osteosarcoma	2	1	0	0	0	0	1	0	0	0	0
Malignant Hydrothorax (Prostate Cancer, mesothelioma and lung cancer)	1	2	0	1	1	6.4±3.2	2	0	1	0	3.4±2.3

Table 3: NK and LAK activity before and after LBP/LAK/IL-2 combination treatment

No.	LAK/IL-2 only group						No.	LBP plus LAK/IL-2 group					
	NK activity (%)			LAK activity (%)				NK activity (%)			LAK activity (%)		
	Before	After	Increase	Before	After	Increase		Before	After	Increase	Before	After	Increase
1	ND	17.3	ND	25.4	28.8	3.4	10	11.2	19.8	8.6	18.4	30.5	12.1
2	15.6	20.5	4.9	28.9	34.1	5.2	11	14.5	25.0	10.5	24.1	36.5	12.4
3	12.0	15.8	3.8	24.1	30.9	5.9	12	10.4	16.9	6.5	20.1	30.0	9.9
4	11.3	13	1.7	21.6	24.5	4.9	13	ND	ND	ND	21.5	30.1	8.6
5	16.3	20.1	3.8	25.8	30.9	5.1	14	11.0	19.1	7.9	18.1	27.6	9.5
6	9.4	14.1	4.7	18.1	22.4	4.3	15	10.9	17.1	6.2	18.1	26.8	8.8
7	9.0	9.3	0.3	12.5	11.1	-1.4	16	19.1	25.7	6.6	27.1	37.8	10.7
8	14.4	18.9	4.5	20.6	26.7	6.1	17	14.5	19.8	5.3	2.0	36.4	15.4
9	14.7	19.8	5.1	24.1	25.2	1.1	18	8.4	14.5	6.1	19.2	30.5	11.3
							19	11.0	15.6	4.5	18.0	ND	ND

Discussion:

Rosenberg et al. discovered the effectiveness of LAK/IL-2 treatment for advanced cancer in 1985. Since then, LAK/IL-2 therapy has been widely studied, although researched dosages have varied widely. Based on animal studies, the U.S. and European countries have tended to favor larger doses of IL-2 ($1 \times 10^5 \cdot \text{kg} \cdot \text{d}^{-1}$). Eastern countries have preferred to use smaller dosages. This study utilized a medium dosage. In previous clinical trials, LAK/IL-2 treatments at this dosage had limited effects. However, LBP combined with LAK/IL-2 therapy significantly increased the effectiveness of the treatment.

The fundamental principle of cancer biotherapy is to destroy tumor cells and help restore natural anti-tumor function in the body. Some active components in herbs possess biological reaction modulation (BRM) properties that are essential in cancer biotherapy.

Geng et al. found that LBP stimulated the T cells to produce IL-2 in the elderly. This may contribute to LBP's ability to enhance the anti-cancer activity of LAK/IL-2. Further studies need to be done to explain the mechanism of BRM in herbs.

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