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Ikuro Kimura\*

Taisuke Ohnoshi†

Yasunari Nakata‡

Kenta Takasugi\*\*

Masafumi Fujii††

Kyoichi Hayashi‡‡

Mikio Kataoka§

Masaharu Sato¶

Ryuji Nishihara||

\*Okayama University,

†Okayama University,

‡Okayama University,

\*\*Okayama University,

††Okayama University,

‡‡Okayama University,

§Okayama University,

¶Okayama University,

||Okayama University,

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## Abstract

Clinical trials of immuno-chemotherapy were conducted on malignant lymphoma patients. Patients during the period from 1972 through 1977 were allocated to two groups retrospectively according to the mode of treatment, i.e., chemotherapy alone (historical control group, 35 patients) and chemotherapy with OK-432 (treated group, 15 patients). Comparisons were made of the two groups, which were homogeneous with regard to induction chemotherapy, maintenance chemotherapy, stage and histologic type of disease. The treated group had a higher remission rate, and a longer remission duration and survival than the control groups, especially in patients with Hodgkin's disease but the difference was not statistically significant owing to the limited number of cases.

**KEYWORDS:** malignant lymphoma, chemotherapy, nonspecific immunotherapy, OK-432

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## IMMUNO-CHEMOTHERAPY OF MALIGNANT LYMPHOMA USING OK-432, A STREPTOCOCCAL AGENT

Ikuro KIMURA, Taisuke OHNOSHI, Yasunari NAKATA,  
Kenta TAKASUGI, Masafumi FUJII, Kyoichi HAYASHI,  
Mikio KATAOKA, Masaharu SATO  
and Ryuji NISHIHARA

*Second Département of Medicine, Okayama University Medical School,  
Okayama 700, Japan*

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*Abstract.* Clinical trials of immuno-chemotherapy were conducted on malignant lymphoma patients. Patients during the period from 1972 through 1977 were allocated to two groups retrospectively according to the mode of treatment, *i. e.*, chemotherapy alone (historical control group, 35 patients) and chemotherapy with OK-432 (treated group, 15 patients). Comparisons were made of the two groups, which were homogeneous with regard to induction chemotherapy, maintenance chemotherapy, stage and histologic type of disease. The treated group had a higher remission rate, and a longer remission duration and survival than the control group, especially in patients with Hodgkin's disease but the difference was not statistically significant owing to the limited number of cases.

*Key words:* malignant lymphoma, chemotherapy, nonspecific immunotherapy, OK-432.

Previous studies with experimental tumor systems have shown that the maximum number of tumor cells killed by the immune systems is not more than  $10^5$ . Immuno-chemotherapy with anticancer agents and immunoajuvant is theoretically alleged to have the following advantages: (a) it is effective against small number of residual tumor cells, especially those in long  $G_1$  through  $G_0$  phase, which cannot be eradicated by anticancer agent alone, and (b) it may protect the host from immunosuppressive toxicity of anticancer agents or restore host immunity. Malignant lymphoma has such high chemosensitivity that intensive chemotherapy brings about complete remission relatively easily. Therefore, patients with malignant lymphoma may be good candidates for immuno-chemotherapy. Beneficial effects of immunotherapy with BCG in patients with malignant lymphoma have already been reported by Sokal *et al.* (1), Thomas *et al.* (2) and Hoetri *et al.* (3). We used streptococcal agent OK-432 (4-6) in immunotherapy of malignant lymphoma patients with the intention of assessing the efficacy of this preparation.

## MATERIALS AND METHODS

*Patients.* Fifty previously untreated patients admitted to the Second Department of Medicine, Okayama University Hospital, from October 1972 through November 1977, were involved in the present study. The diagnosis of malignant lymphoma was confirmed by biopsy, and all patients were staged by the routine procedures proposed in the Ann Arbor Symposium (7). As indicated in Table 1, 15 of 50 patients were treated with immuno-chemotherapy combined with OK-

TABLE 1. CHARACTERISTICS OF PATIENTS

|                           | BVCP/B-COP plus OK-432 | BVCP/B-COP alone |
|---------------------------|------------------------|------------------|
| No. of cases (No. of men) | 15(11)                 | 35(26)           |
| Median age (Range)        | 61(18-77)              | 46(14-70)        |
| Type of lymphoma          |                        |                  |
| Hodgkin's disease         | 5                      | 10               |
| Reticulum cell sarcoma    | 7                      | 19               |
| Lymphosarcoma             | 3                      | 6                |
| Induction chemotherapy    |                        |                  |
| BVCP                      | 9                      | 18               |
| B-COP                     | 6                      | 17               |

432 while the remaining 35 patients received chemotherapy alone as a historical control. Two additional relapsed patients who had had prior chemotherapy but were not included in the comparative study were evaluated for several immune parameters while receiving OK-432 alone.

*Treatment.* As shown in Fig. 1, induction chemotherapy consisted of the four-

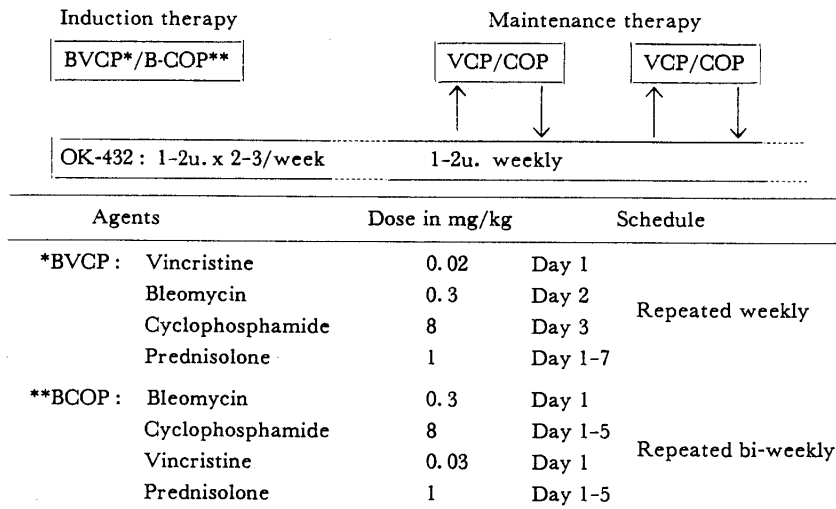


Fig. 1. Treatment schedule of immuno-chemotherapy in malignant lymphoma patients.

drug combination; vincristine (VCR), bleomycin (BLM), cyclophosphamide (CPA) and prednisolone (PS), given on two different treatment schedules: (a) BVCP: VCR, 0.02 mg/kg, day 1; BLM, 0.3 mg/kg, day 2; CPA, 8 mg/kg, day 3; PS, 1 mg/kg, day 1 to 7; weekly, and (b) B-COP: BLM, 0.3 mg/kg, day 1; CPA, 8 mg/kg, day 1 to 5; VCR, 0.03 mg/kg, day 1; PS, 1 mg/kg, day 1 to 5; biweekly. After one of the two induction chemotherapies defined above was given for 8 to 12 courses, complete responders received maintenance therapy with a three-drug combination of CPA, VCR and PS, in intermittent doses. OK-432 administered concomitantly with induction chemotherapy as 1 to 2 units intramuscularly 2 to 3 times a week, and subsequently at the same dosage level 1 to 2 times a week during the maintenance phase. Six patients were given OK-432 for 2 weeks prior to induction chemotherapy and several immune parameters determined serially.

*Measurement of immune parameters.* The following immune parameters were evaluated serially during the period of the study; (a) PPD skin test and PHA skin test; (b) *in vitro* blastogenic activity of lymphocytes by PHA, determined by whole blood technique of Pellegrino *et al.* (8); (c) T and B lymphocyte counts; (d) monocyte counts; and (e) chemotactic activity of monocytes, determined in accordance with the method of Snyderman *et al.* (9) using zymosan-activated sera as the chemoattractant.

## RESULTS

A tendency for higher counts in T lymphocytes and monocytes was observed in 8 patients receiving OK-432 alone. However, *in vitro* blastogenic activity of lymphocytes by PHA was unchanged in most of these patients. No statistically significant changes were observed in other parameters due to the limited number of patients. Significant tumor regression was noted in 2 of the 8 patients on OK-432 alone (Figs. 2 and 3). In a patient with Hodgkin's disease

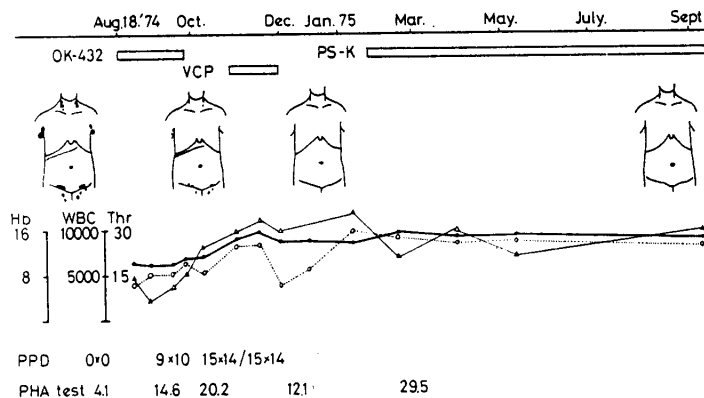


Fig. 2. Effect of OK-432 treatment prior to induction chemotherapy in 73 year old male with Hodgkin's disease.

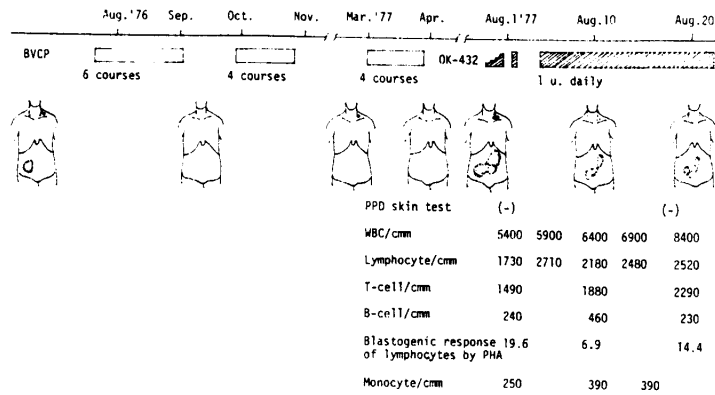


Fig. 3. Effect of OK-432 treatment prior to induction chemotherapy in 19 year old female with reticulum cell sarcoma.

(Fig. 2), enhancement of PPD skin reaction and return to normal of *in vitro* lymphocyte blastogenic activity by PHA were observed with tumor regression. In a patient previously treated for reticulum cell sarcoma (Fig. 3), increased monocyte and lymphocyte counts were noted but no significant changes were observed in either the PPD skin reaction or lymphocyte blastogenic activity.

Comparisons were made of complete remission rate and remission duration between patients treated with immuno-chemotherapy (treated patients) and patients treated with chemotherapy alone (control patients) (Table 2). Among

TABLE 2. RESPONSE RATE AND REMISSION DURATION IN PREVIOUSLY UNTREATED PATIENTS: BVCP/B-COP ALONE VS. BVCP/B-COP PLUS OK-432

| Cell type              | OK-432 | No. of cases | No. of complete responders (%) | Remission duration (Mos.) |        |
|------------------------|--------|--------------|--------------------------------|---------------------------|--------|
|                        |        |              |                                | Range                     | Median |
| Hodgkin's disease      | Yes    | 5            | 5(100)                         | 3 -54+                    | 15     |
|                        | No     | 10           | 6( 60)                         | 4 -51+                    | 6.3    |
| Non-Hodgkin's lymphoma | Yes    | 10           | 6( 60)                         | 4+ -39+                   | 6.5    |
|                        | No     | 25           | 15( 60)                        | 3 -43+                    | 6.0    |

patients with Hodgkin's disease, treated patients had more favorable figures in both remission rate and remission duration than control patients, but the difference was not statistically significant owing to the limited number of cases. Little difference was noted between the treated and control groups of non-Hodgkin's lymphoma patients in these respects. Next, the survival times of patients with stage III or IV Hodgkin's disease were compared in the treated and control

groups. Fig. 4 shows that treated patients had longer survival times than control patients but that the difference was not significant because of the small number of cases available. There was no noticeable difference in survival time between the two groups for patients with non-Hodgkin's lymphoma.

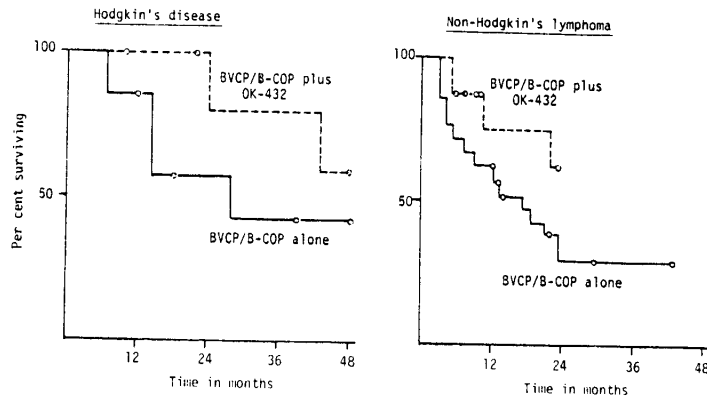


Fig. 4. Survival of stage III and IV lymphoma patients: BVCP/B-COP alone vs. BVCP/B-COP plus OK-432.

TABLE 3. CHANGES IN PPD SKIN REACTION OF MALIGNANT LYMPHOMA PATIENTS ACCORDING TO THERAPY RESPONSE: CHEMOTHERAPY ALONE VS. CHEMOTHERAPY PLUS OK-432

|                     | With OK-432           |                            | Without OK-432        |                            |
|---------------------|-----------------------|----------------------------|-----------------------|----------------------------|
|                     | No. of cases examined | No. with enhanced reaction | No. of cases examined | No. with enhanced reaction |
| Complete responders | 11                    | 5                          | 15                    | 7                          |
| Partial responders  | 2                     | 1                          | 14                    | 3                          |
| Non-responders      | 2                     | 0                          | 2                     | 0                          |
| Total               | 15                    | 6(40.0%)                   | 31                    | 10(32.3%)                  |

Serial determination of immune parameters during the course of treatment showed a recovery toward normal of the PPD skin reaction and *in vitro* lymphocyte blastogenic activity by PHA (Fig. 5) in complete responders, but the difference between treated and control patients was not statistically significant. Data on monocyte counts and chemotactic activity of monocytes were obtained in only some cases, and hence are not presented separately for treated and control patients. Partial responders and nonresponders showed a decrease in monocyte counts; complete responders tended to show increased values (Fig. 6). The

chemotactic activity of monocytes diminished with progress in chemotherapy, but returned to normal when complete remission was achieved (Fig. 7).

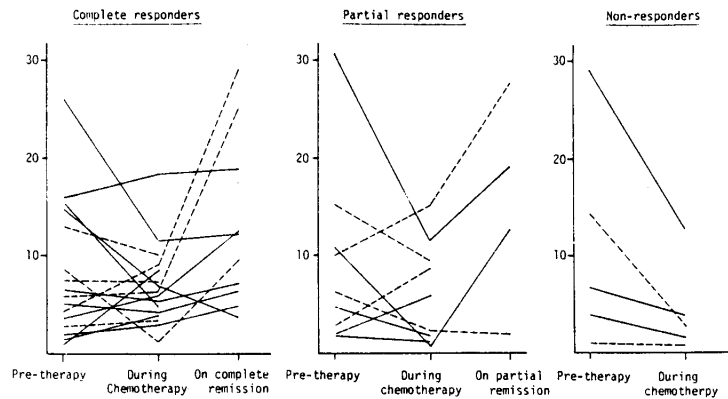


Fig. 5. Changes in lymphocyte blastogenic activity by PHA according to therapy response. Broken lines represent cases treated by immuno-chemotherapy.

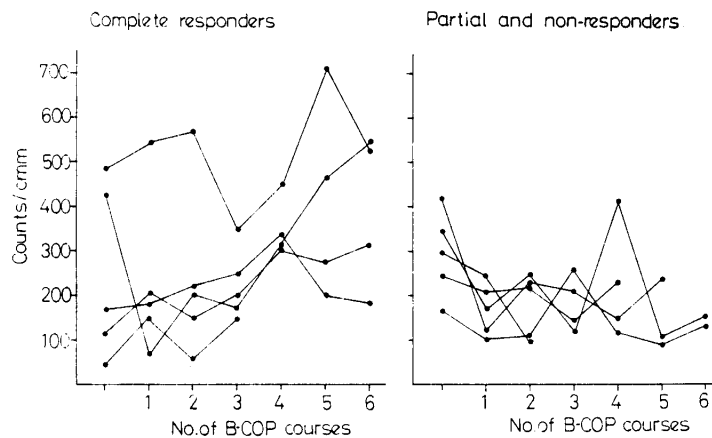


Fig. 6. Changes in monocyte counts in malignant lymphoma patients during B-COP therapy.

#### DISCUSSION

Our previous results with OK-432 in experimental tumor systems (5) and lung cancer patients (6) indicated that the drug is an active immunoadjuvant. The use of OK-432 alone in malignant lymphoma patients was shown to be associated with an increase in T lymphocyte and monocyte counts. *In vitro* blastogenic activity of lymphocytes, on the other hand, did not change distinctly in the drug treated malignant lymphoma patients as we observed previously



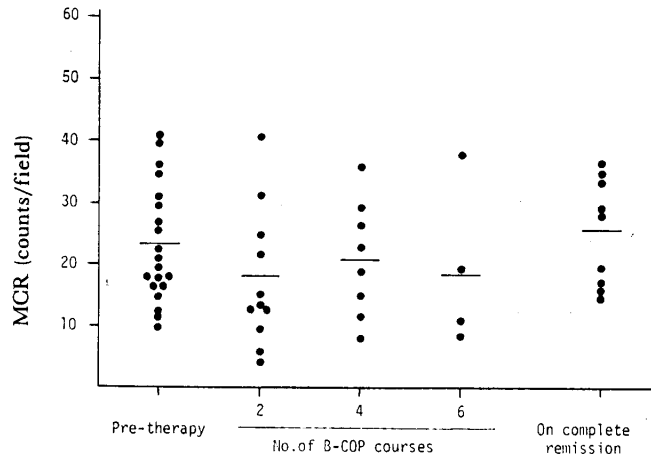


Fig. 7. Monocyte chemotactic activity in malignant lymphoma patients during B-COP therapy.

with lung cancer patients. In some patients with malignant lymphoma, however, OK-432 medication as sole therapy was associated with tumor regression and recovery toward normal of immune parameters. This observation, even if not valid for all patients with large tumors, may be considered evidence that the drug produces some favorable effects on the immune systems of patients with malignant lymphoma. The findings also suggest that OK-432, when used in patients with malignant lymphoma who are freed from a large tumor burden, *i. e.*, in a state of complete remission, may produce an even more favorable effect on the host immune systems.

The treated and control groups employed in the present study may be considered homogeneous with regard to not only induction chemotherapy, but also the stage and histologic type of disease. The results indicated the patients with Hodgkin's disease in the treated group had a higher remission rate, and a longer remission duration and survival than those in the control group. The reason immuno-chemotherapy produced better clinical effects in patients with Hodgkin's disease may be that Hodgkin's disease has higher chemosensitivity and a slower growth than non-Hodgkin's lymphoma.

OK-432 treatment was begun early in the induction chemotherapy, and immune parameters were evaluated serially during the subsequent period of treatment in some cases. Recovery of immune parameters was noted in complete responders. Since there were no substantial differences between treated and control patients in these parameters, however, it seems that the favorable changes in immune parameters are attributable to the lessening of tumor burden through chemotherapy rather than to OK-432. The present results did not

permit any definitive statements about OK-432 restoration of the host immune responses or protection of the host immunity from the immunosuppressive toxicity of anticancer agents. The results of the present study, however, indicate that immunochemotherapy with OK-432 and the four-drug regimen of VCR, BLM, CPA and PS seems an effective therapeutic means of treating malignant lymphoma patients. Further studies are necessary to determine the optimal conditions for OK-432 therapy, including the route of administration, dose level and timing of treatment. Before these are clarified, extensive evaluation of immune parameters will have to be made. Then, randomized studies can be undertaken for objective assessment of the efficacy of OK-432.

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