

Effect of Intravenous Immunostimulant (OK-432)

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Received for publication, December 26, 1987

ABSTRACT : To clarify the effectiveness of OK-432, immunopotentiator was intravenously used. The tumor-bearing host was apparently stimulated via NK activity and increased the number of T cells and B cells. It helps immunodepressed host to level up quickly.

However, further effective immunostimulation beyond the normal range could not be expected. A new combined method should be found to be immunopotentiated even for the uncompromized host. Recently, a new immunostimulant derived from streptococcus, OK-432, was widely used for the treatment of cancer in Japan. The cytostatic and cytotoxic actions of OK-432 for cancer cells have been made clear by in vitro and vivo study. It, however, is not clear as to how the developing immunostimulated states differ on the condition of admistering route, dosis or time-intlrvl.

In this series, the effects of intravenous administration of OK-432 as the host immunostimulator were compared with those of conventional subcutaneous administration.

MATERIAL AND METHOD

Forteen patients with cancers of the digestive tract, in stage II or III, in whom a curative operation was assumed feasible preoperatively, including eight gastric cancers (stage II in two, stage III in six) and six colon cancers (stage III in all), were eligible to this study. Changes in lymphocyte subsets were analyzed at the third day after 0.1 KE intravenous administration of OK-432. Three times intravenous administration of 0.2 KE OK-432 was added in nine patients, consisted of five gastric cancers (stage II in one, stage III in four) and four colon cancers (stage II in one, stage III in three, to study the effects of OK-432 as an immunopotentiator. Cell preparation the blood samples were taken three days before assessment of lymphocyte subset.

Peripheral blood mononuclear cell (PBM) were isolated by Ficoll-Hypaque density gradient sedimentation. The cells were suspended in the RPMI 1640 medium containing 10% pooled human AB serum, supplemented with penicillin 100 μ /ml and streptomycin 100 μ g/ml.

Lymphocyte subsets : Analysis of surfactant markers on lymphocyte subpopulations by monoclonal antibodies was performed according to the method of STEPHAN *et al.*¹⁾ Analysis of fluorescence staining of cells was performed by means of single and two-color flow-cytometry using the FACS intervenous flow-cytometry system. Monoclonal antibodies used for phenotyping were CD3, CD4, CD8, CD20, CD11, Leu7, CD16, OK1a1, HLA-DR. Administration of OK-432 0.1 KE of OK-432 was intravenously given. At the third day, lymphocyte subsets were tested. In this preliminary study, however, it was found that an insufficient state of

immunostimulation has generated. Therefore, 0.2 KE of OK-432 were given three times at an interval on three days as shown in Fig. 1.

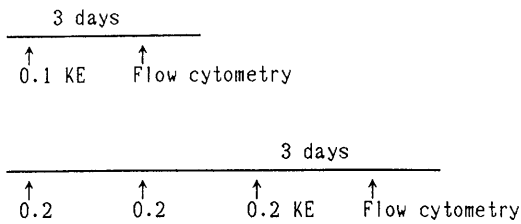


Fig. 1. Schema of this study, upper : once 0.1 KE OK-432 1V administration, thereafter 3 days later flow cytometric analysis was done. lower : 3 times 0.2 KE OK-432 1V at an interval of 3 days

RESULTS

The results of intravenous administration of 0.1 KE of OK-432 were shown in Table 1. Each subset of lymphocytes did not change with varying variety. However, an increase in Leu7, and CD16 positive cells was remarkably noted, suggesting an action of immunopotential.

Table 1. Changes in subset of lymphocytes 3 days after 0.1 KE OK-432 1V administration

	n	Percent positive cells	
		before	3 days after
CD 3	14	64.4 ± 8.0	62.9 ± 9.1
CD 4	14	43.0 ± 7.2	40.7 ± 9.8
CD 8	14	27.3 ± 8.7	27.6 ± 7.8
CD 20	14	16.7 ± 6.3	13.3 ± 4.6
CD 11	14	28.8 ± 8.7	30.8 ± 10.8
Leu 7	14	13.8 ± 7.8	16.6 ± 8.1
	12	11.6 ± 5.4	16.0 ± 7.7* ¹
CD 16	14	17.6 ± 8.6	18.4 ± 10.9
	9	9.2 ± 4.1	16.4 ± 8.6* ²
OKIal	14	22.9 ± 8.4	21.5 ± 7.3
HLA-DR	14	28.7 ± 8.2	27.0 ± 5.0

On the other hand, when 0.2 KE of OK-432 intravenous was given three times as shown in Table 2, the subsets of lymphocytes, CD20, CD 11, Leu7, OK1a1, HLA-DR and CD8, CD11 and CD4, 2H4 were markedly increased in number. Three times 0.2 KE OK-432 intravenous administration facilitated potentially an in-

Table 2. Changes in subset of lymphocytes 3 days after 3 times 0.2 KE OK-432 1V administration at an interval of 3 days

	n	Percent positive cells	
		before	3 days after
CD 3	9	66.2 ± 10.1	65.8 ± 14.3
CD 4	9	42.5 ± 7.6	44.4 ± 11.9
CD 8	9	26.8 ± 5.1	27.8 ± 6.1
CD 20	9	10.8 ± 2.6	13.1 ± 4.5* ¹
CD 11	9	32.4 ± 9.2	41.6 ± 7.2* ²
Leu 7	9	19.6 ± 11.5	24.0 ± 10.4* ³
CD 16	9	17.3 ± 13.1	18.4 ± 9.3
OK Ial	9	23.1 ± 9.8	34.4 ± 7.6* ⁴
HLA-DR	9	21.4 ± 7.1	32.3 ± 7.2* ⁵
CD 8 ⁺ CD 11 ⁺	5	8.0 ± 3.7	8.6 ± 4.2
CD 8 ⁺ CD 11 ⁻	5	17.0 ± 6.4	15.2 ± 6.6* ⁶
CD 4 ⁺ 4 B 4 ⁺	5	24.8 ± 7.5	22.8 ± 9.5
CD 4 ⁺ 2 H 4 ⁺	5	14.4 ± 5.1	19.9 ± 8.3* ⁷

crease in subpopulation of lymphocytes much more than 0.1 KE administration. An increase in activated T.B and NK cells was obviously noted to some extent in proportion to a dosis and an interval times of OK-432 administered. An increase in the population of these cells closely relates to the dosis, the frequency and the time-interval of OK-432 administration to immunodepressed tumor-bearing host. From an analysis by using two-color flow cytometry method, the effect of OK-432 on activation of lymphocytes was much more apparently shown than when compared with non-OK-432 as indicated in Fig. 2 on the condition of 0.2 KE OK-432 intravenous administration three times per three days intervals.

DISCUSSION

Even in intravenous administration of OK-432, the amount and frequency directly related to the stimulating effect on generation of activated subpopulation of T cells in PBM.

GRIMM *et al*²⁾ showed that OK-432 induced cytotoxic cells after in vitro activation of PBM with the agent exhibited properties identical to LAK cells. Needless to say, LAK cells represent cytotoxic action for cancer cells, which are effectively acting as not only prevention of spread of cancer extension but also elimination of the tumor size. It is no

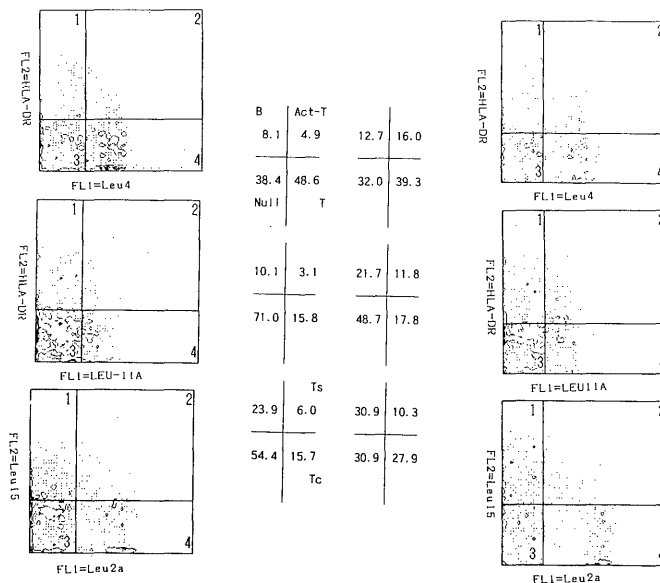


Fig. 2. Analysis of activation of lymphocytes by using two color flow cytometry method

doubt that OK-432 administration promotes generation of LAK cells. As a result, the immunopotentiated host may help facilitate cancer therapy to give cytotoxic damage to cancer cells. It is widely accepted as previously cited by Bush's report³⁾ that a malignant human tumor regressed when the patient contracted erysipelas. Even though OK-432 were given via intravenous route, the more the amount and the frequency of OK-432 were increased, the more generation of subset of activated lymphocytes in PBM was enhanced.

Interestingly enough, OK-432 administration arose generation of HLA-DR(+) cells, which were composed of activated T, activated B and CD16 (Leu11a).

In view of host-mediated immunopotentiality by OK-432, OK-432 intervenous administration effectively promotes the activation of NK cells and also increases the number of activated T and B cells to make up for a depressed immune state of tumor-bearing host.

On the other hand, it is emphasized that OK-432 reveals a direct cytotoxic effect on malignant cells. In Japan endoscopic direct injection of OK-432 around cancer lesions has been applied for the treatment of cancers of the stomach and the lung.⁴⁾⁵⁾

Some reports cytologically evidenced direct cytotoxic effect on malignant cells.⁶⁾⁷⁾ For this advantage of this agent, OK-432 was used locally as a treatment of malignant pleural effusion and ascitic fluid, expecting the direct cytotoxic action of OK-432.

Cell damage to tumor cells by exposure to OK-432 is apparently presented in comparison with that prior to exposure to OK-432 by many investigators⁶⁾.

In contrast, it is defined that effectiveness of OK-432 in vivo observation closely correlates with the response to SU-PS skin test⁸⁾⁹⁾. SU-PS contains streptococcal protein. Therefore, the response to SU-PS skin-test reflects the reaction to a type of delayed hypersensitivity, showing a sensitized state to the antigen associated with streptococcal protein. It is clear that the stronger the positive SU-PS skin test, the more the immune response of a host to cytotoxicity of cancer cells is enhanced.

In conclusion, OK-432 is effective in enhancing cell-mediated immunity to tumor-bearing host as well as in facilitating direct cytotoxicity to cancer cells when directly exposing to cancer cells.

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