

Acta Medica Okayama

Volume 28, Issue 6

1974

Article 5

DECEMBER 1974

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Abstract

The streptococcal preparation, OK.432, with predominant host-mediated mode of action, was studied. By giving OK-432 to mice intraperitoneally prior to transplantation of Ehrlich carcinoma, a host-mediated action to increase life-span was clearly confirmed. Pretreatment with OK-432 was also effective against the development of Rauscher leukemia. The host-mediated action of OK-432 varied with the interval between its pretreatment and the inoculation of tumor cells. The effect was most marked when the transplant was performed immediately after the pretreatment, and became less marked when the transplant was made one week and two weeks after pretreatment. The host-mediated action can be observed even with a single dose of pretreatment, and becomes more potent as pretreatment was given repeatedly. The host-mediated action was weakened by concomitant pretreatment with cyclophosphamide or roentgen irradiation, and the mechanisms of such action was supposed to be associated with the function of the reticulo-endothelial system.

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Acta Med. Okayama 28, 423—431 (1974)

**STUDIES ON THE HOST-MEDIATED ACTION OF
THE STREPTOCOCCAL PREPARATION, OK-432,
IN CANCER CHEMOTHERAPY**

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Received for publication, May 7, 1974

Abstract: The streptococcal preparation, OK-432, with predominant host-mediated mode of action, was studied. By giving OK-432 to mice intraperitoneally prior to transplantation of Ehrlich carcinoma, a host-mediated action to increase life-span was clearly confirmed. Pretreatment with OK-432 was also effective against the development of Rauscher leukemia. The host-mediated action of OK-432 varied with the interval between its pretreatment and the inoculation of tumor cells. The effect was most marked when the transplant was performed immediately after the pretreatment, and became less marked when the transplant was made one week and two weeks after pretreatment. The host-mediated action can be observed even with a single dose of pretreatment, and becomes more potent as pretreatment was given repeatedly. The host-mediated action was weakened by concomitant pretreatment with cyclophosphamide or roentgen irradiation, and the mechanisms of such action was supposed to be associated with the function of the reticulo-endothelial system.

It has been gradually shown that OK432, (1, 2) a streptococcal preparation developed in Japan, has a specific mode of action. We have gone a step further towards the treatment of malignant tumors, especially leukemia to study combination therapy of OK-432 with the so-called anti-cancer agents (4, 5), and shown that combination therapy produces remarkable effect. In these studies, we noted the fact that the agent hardly showed a cytotoxic activity on tumor cells *in vitro* while it was moderately effective on tumor-bearing mice, and that the response of each patient to OK-432 was slightly dose-related in clinical use. The idea to use a streptococcal preparation came primarily from the fact that regression of malignant tumors was seen during the attack of serious infection by hemolytic streptococci (6, 7, 8); it appears that OK-432 may possess a host-mediated action that has not been found with other anti-cancer agents. Our present report concerns the results obtained so far from our studies as to the presence or absence of host-mediated action and its mechanism.

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MATERIALS AND METHODS

OK-432: This agent was prepared from a low-virulent strain *Su* of *streptococcus haemolyticus* established by OKAMOTO *et al.* (1, 2). One KE (Klinische Einheit, *i. e.*, unit) contains 0.1 mg of prepared hemolytic streptococci in dry weight.

Animal: Six week-old male ICR mice were employed for Ehrlich carcinoma, and also six week-old male CB mice for Rauscher leukemia.

Ehrlich ascitic tumor: Six to seven days after intraperitoneal (*i. p.*) transplantation, tumor cells were harvested and washed once with ice cold phosphate-buffered saline (PBS) solution, and subsequently the cell number was adjusted to 10^1 , 10^2 , 10^3 or 10^4 in 0.1 ml, which was inoculated *i. p.*. As for intravenous (*i. v.*) inoculation, tumor cells were washed 3 times in order to avoid aggregation of tumor cells and 2×10^6 cells in 0.1 ml was inoculated into the tail vein.

Rucher leukemia: Mice were inoculated with a dose of Rauscher leukemia viruses (RLV) in 0.2 ml of the supernatant containing 20% spleen homogenate.

Pretreatment with OK-432: Pretreatment was made *i. p.* or subcutaneously (*s. c.*) with 1KE/mouse of OK-432 for various periods prior to the tumor transplantation as indicated in the results. To control group 0.1 ml of isotonic saline solution was given for the same periods as experimental groups.

India ink clearance: To three groups consisted of 12 mice in each group, 1KE/mouse of OK-432 alone or 0.5 mg/kg, body weight, of Mitomycin C (MMC) combined were administered for 10 days. On the days 1 and 10 after completion of the treatment mice were sacrificed, and India ink clearance was tested according to the method reported by WATANUKI (9).

Hemolytic plaque forming cell (HPFC) count: This was done according to CUNNINGHAM (10). Treatment with OK-432 was made 24 hr before the *i. p.* immunization with sheep red blood cells, and HPFC count was studied five days after the immunization.

RESULTS

1. *Effects of pretreatment on Ehrlich carcinoma.*

OK-432, Cyclophosphamide (CPA) or MMC was given *i. p.* for 5 days and Bleomycin once for all, and 5 days later 2×10^6 of tumor cells were inoculated *i. v.* via the tail vein. Through observation of pulmonary metastasis two weeks after inoculation, pretreatment with OK-432 was found to have inhibited the metastasis, in contrast to pretreatment with other anticancer agents, such as CPA, MMC and Bleomycin, which enhanced the metastasis (Fig. 1).

Action of OK-432 was studied further by changing the interval between pretreatment and *i. v.* transplantation of Ehrlich carcinoma in three ways (Fig. 2); on the following day and one and two weeks after pretreatment. The group receiving *i. v.* inoculation on the next day of pretreatment showed an excellent 50-day-survival rate as compared with the control group. Prolongation of life-span to some degree, but less than the above, was also noted

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in the group that was inoculated one week later. In the group transplanted two weeks after pretreatment, the survival rate was similar to that of control without showing any life prolongation.

Subsequently, the effect of pretreatment with OK-432 injected i. p. on Ehrlich carcinoma inoculated also i. p. was studied (Fig. 3). Although i. p. transplantation of tumor cells as many as 10^6 was not affected by pretreatment with OK-432, influence of pretreatment was noted when 10^4 cells were inoculated i. p.. This tendency for prolonging life-span with pretreatment became marked as the inoculation size of cells was decreased in the order of 10^3 , 10^2 and 10^1 , suggesting the presence of a host-mediated effect on the i. p. transplantation as in the case of i. v.. A similar tendency was also noted in the s. c. pretreatment with OK-432. The influence of a repeated pretreatment was studied by examining the survival rate in three groups of mice that were treated for one, 7 and 14 consecutive days, followed by i. p. inoculation of the tumor cells. The life-prolongation was appreciable even with pretreatment for one day, and the effect was increased as pretreatment was carried out repeatedly (Fig. 4).

The effects of pretreatment combined with OK-432 and CPA prior to the i. v. transplantation of tumor cells were then studied (Fig. 5). With OK-432 alone the same results as described above were obtained again, and with CPA alone survival shorter than the controls was noted. In contrast to pre-

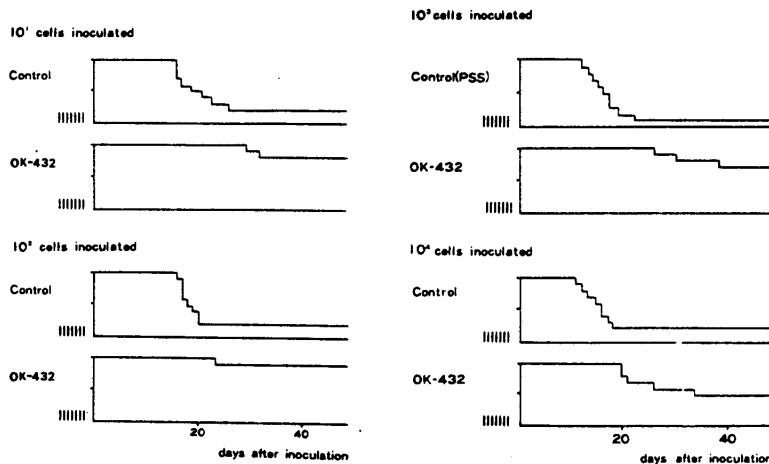


Fig. 3. Effect of pretreatment with OK-432 on i. p. inoculated Ehrlich carcinoma

To each group consisting of 10 mice, the same pretreatment as Fig. 2 was done. Two days later, 10^1 , 10^2 , 10^3 or 10^4 of tumor cells were inoculated i. p., followed by survival.

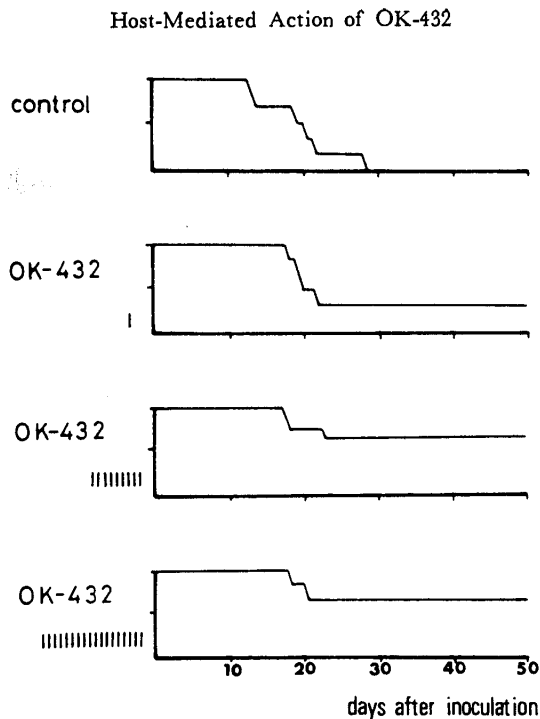


Fig. 4. Dose frequency of pretreatment with OK-432 and effect on Ehrlich carcinoma

Each group consisting of 10 mice was pretreated i. p. with OK-432 for one, 7 and 14 days. Two days later 10^4 of tumor cells were inoculated i. p., followed by survival.

treatment with OK-432 which produced favorable prolongation of life, CPA decreased the effect of OK-432 when used concomitantly.

The same results were also obtained with whole-body irradiation; the irradiation weakened the action of OK-432, and simultaneously, the effect of irradiation on mice was somewhat lessened by the use of OK-432. It was shown, therefore, that the host-mediated action of OK-432 can hardly be exerted under immuno-suppressed conditions, raising an important problem in cancer chemotherapy.

2. *Effects of pretreatment on Rauscher leukemia.*

When OK-432 was administered prior to the inoculation of RLV, an inhibition was apparently indicated by spleen weights and peripheral nucleated cell counts, and similar results were also obtained when OK-432 was given before as well as after the inoculation, whereas the effects of OK-432, when given after the inoculation, did not differ much from the controls (Fig. 6). In other words, the influence of the host-mediated action of OK-432 was noted at the onset of Rauscher leukemia. In addition, it was demonstrated

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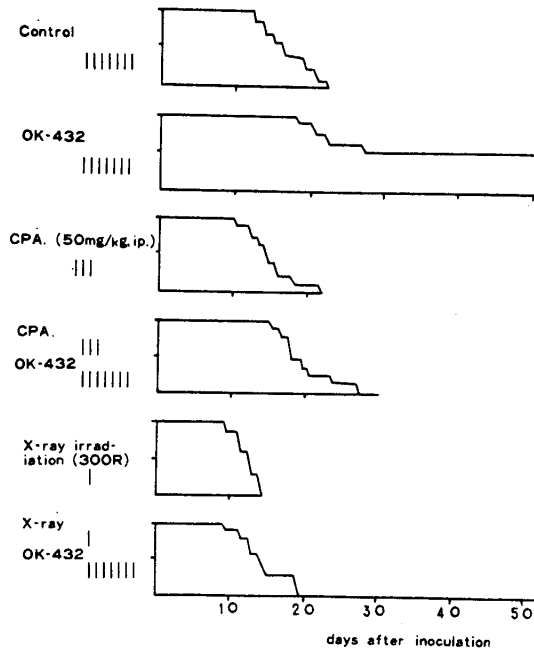


Fig. 5. Effect of pretreatment with OK-432 and CPA or whole body X-ray irradiation on i. v. inoculated Ehrlich carcinoma

Pretreatment was started at day 9 prior to i. v. inoculation of 2×10^6 of tumor cells. OK-432 for 7 days, CPA for 3 days and whole body X-ray irradiation once for all.

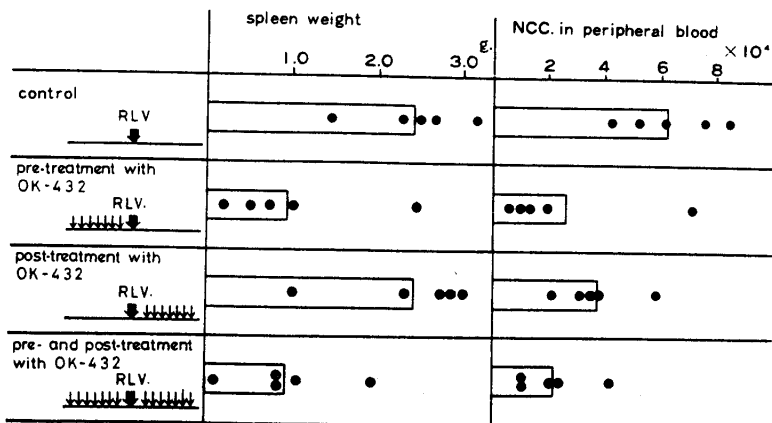


Fig. 6. Effect of OK-432 on Rauscher leukemia

Spleen weight and peripheral nucleated cell counts were studied 3 weeks after inoculation.

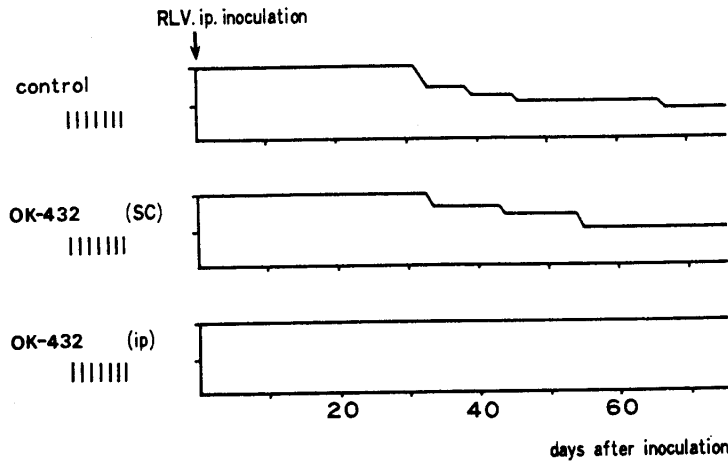


Fig. 7. Effect of pretreatment with OK-432 on survival of Rauscher leukemia mice

To each group consisting of 12 mice, OK-432 was given i. p. or s. c. for 7 days. One week later, RLV was inocuated i. p., followed by survival.

that pretreatment with OK-432 exerted some influence on the survival in Rauscher leukemic mice ; this is more predominant when it is pretreated, i. p. more than when done s. c. (Fig. 7).

3. *The mechanism of host-mediated action.*

This was studied based on the function of reticulo-endothelial system (Fig. 8). The results indicated that reticulo-endothelial system function was enhanced by the administration of OK-432, whereas the combined use of OK-432 with MMC somewhat lessened the the diminution of the function. By studying the effect of OK-432 on the antibody-forming capacity by means of the HPFC count, OK-432 showed a slight tendency, not significant, to increase HPFC in some cases (Table 1).

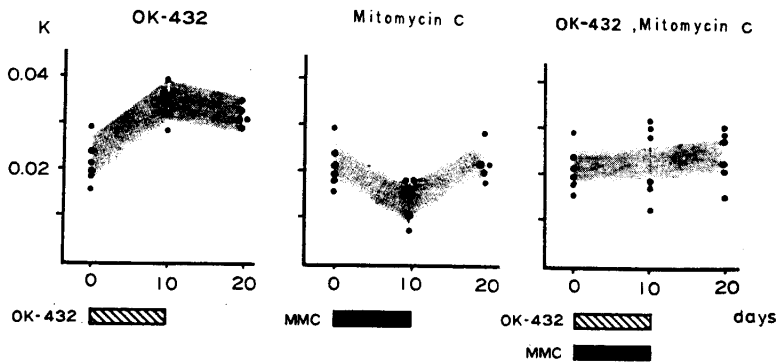


Fig. 8. Effect of OK-432 and/or MMC on India ink clearance

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TABLE 1 EFFECT OF OK-432 ON ANTIBODY-FORMING CELLS IN SPLEEN.

Treatment	No. of mice studied	total spleen cells ($\times 10^6$)	HPFC/ 10^6 spleen cells	HPFC/spleen ($\times 10^4$)
control	5	144 (110~165)	1271 (968~2067)	18.1 (15.0~26.9)
OK-432 0.5 ^{KE}	5	168 (115~220)	1479 (982~2337)	24.1 (13.8~31.2)
OK-432 5 ^{KE}	5	191 (170~210)	1135 (753~1555)	21.5 (14.7~30.3)

DISCUSSION

A streptococcal preparation, OK-432, developed by OKAMOTO *et al.* (1, 2) was prepared from the fact that regression of malignant tumors had often been seen during the attack of serious infections (6, 7, 8). As stated above, OK-432 is unique in its pharmacological status and it has a mode of action that is rather difficult to elucidate only in terms of its direct effect on tumor cells in contrast to the conventional series of anti-tumor agents. In studying its influence on the host, apparent life-prolonging effects were observed in mice with transplanted tumors when it was given *i. p.* or *s. c.* as a pretreatment, indicating the presence of host-mediated action. It was shown that this action could be seen even with a single dose and increased as the medication was given repeatedly, maintaining a residual effect decreasing gradually even at about two weeks after withdrawal of medication. A combined use of CPA with OK-432 at pretreatment weakened the host-mediated action and similar results were also obtained by roentgen irradiation. Furthermore, OK-432, when used prior to inoculation of RLV, was found effective against the development of leukemia.

Trigger mechanisms for such effects were revealed partially to the extent that OK-432 stimulated the function of the reticulo-endothelial system but hardly influenced HPFC when tested to observe antibody forming capacity. This indicated that OK-432 possesses a hostmediated anti-tumor action, even though there remains a problem as to whether or not the action has a specific reaction to immunological mechanism.

Systemic and topical administration are recommended as to the application of this drug on man, and KUROKAWA *et al.* (11) described their clinical trial with OK-432 alone in detail especially on the patients with carcinoma. We also experienced (3) a clinical trial on leukemia patients with OK-432 alone. As there was a certain limit of effects for using OK-432 alone, especially given systemically, we attempted (4, 5) a combined use of OK-432 with other anti-cancer agents, and succeeded in obtaining favorable effects. HATTORI *et al.* (12) also obtained similar combined effects by the topical use of OK-432 with systemic use of MMC. As indicated in our experimental

studies (5), the best results were obtained when both OK-432 and anti-cancer agents were given i. p., and OGAWA *et al.* (13) also noted its effectiveness in cancerous pleurisy by infusing it intrathoracically. These facts indicate a possibility of the action of OK-432 through its direct contact with tumor cells. On the other hand, relationship hardly observed between the response to OK-432 and the dose administered in clinical use (3, 11). In addition, the effect of OK-432, when used experimentally, is produced more readily in *in vivo* than in *in vitro*. These facts lead us to postulate the presence of a mode of action other than a direct action and indicate that many other problems exist further with regard to the mechanism of action of OK-432.

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