EFFECTS OF STREPTOCOCCAL PREPARATION OK-432 ON CHEMICALLY-INDUCED INTESTINAL CARCINOMA IN RATS*1

HIDEHITO ICHIHASHI, HAJIME KUZUYA, and TATSUHEI KONDO

*The Second Department of Surgery, Nagoya University School of Medicine*2*
(Director: Prof. Tatsuhei Kondo)

ABSTRACT

Intestinal tumors were induced by repeated subcutaneous injection of 1,2-dimethylhydrazine in Donryu strain rats for 28 weeks. A streptococcal preparation, OK-432 was administered at different times of the tumor induction. Between the OK-432 treated and the untreated groups no significant difference was observed in the incidence of intestinal tumors, the total number of tumors per rat, the location of tumors, or the survival time. Though low incidence of extra-intestinal malignancies in the OK-432 treated animals seemed to be the only positive finding, it was not statistically significant.

INTRODUCTION

OK-432 prepared from *Streptococcus hemolyticus* by Okamoto *et al.*[1)2)] has been reported to have both the direct cytocidal and indirect host-mediated anticancer activity and to inhibit the growth of transplanted tumors.[1)3)4)]

Chemically induced primary autochthonous large bowel cancer in rats is similar to that in man and is resistant to chemotherapeutic agents. The aim of the present work was to investigate whether OK-432 would inhibit the carcinogenesis of 1,2-dimethylhydrazine (DMH) in the bowel of rats.

MATERIALS AND METHODS

Animals

Male Donryu rats used in this study were fed a synthetic diet(MR-2), given tap water *ad libitum.*

Tumor Induction

1,2-dimethylhydrazine (DMH) obtained from Tokyo Kasai Ind. Ltd., Tokyo, Japan, was freshly dissolved in 8.3 ml of a distilled water, then neutralized by 1.5 ml of sodium bicarbonate after adding 5 mg of ethylene diaminotetraacetate for stabilization. DMH in a dose of 25 mg/kg were injected subcutaneously once a week for 28 weeks in order to induce intestinal tumors.

Treatment with OK-432

In Experiment I, one group (Group 1) of rats received subcutaneous injection of 0.2 KE

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*2 65 Tsurumai-cho, Showa-ku, Nagoya 466

市橋秀仁・葛谷　元・近藤達平

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of OK-432 twice a week for 38 weeks. The drug administration was started on the same day as DMH. Other four groups received 1 KE of OK-432 twice a week for 4 weeks. The starts of the drug administration were different in four groups; one from the 2nd month (Group 2), one 4th (Group 3), one 6th (Group 4), one 8th after the first injection of DMH (Group 5). In Experiment II, 25 KE of OK-432 was given once a week for 3 months. The starting day was on the 4th month after the first administration of DMH.

Treatment with Ftorafur

In Experiment II, 1-(2-tetrahydrofuryl)-5-fluorouracil (Ftorafur) was given by mouth every day in a daily dose of 30 mg/kg for 3 months, starting 4 months after the first injection of DMH. The drug was freshly dissolved in drinking water and *given ad libitum.*

Treatment with Syngeneic Tumor Extract

About 1 g of cancer tissue obtained from several intestinal tumors was homogenized with glass homogenizer. Then, the homogenate was centrifuged at 15000 rpm for 30 min. The supernatant was mixed with equal volume of Freund incomplete adjuvant and 0.8 ml of the mixture was injected intramuscularly 3 times at 3-week intervals, starting 2 months after the first injection of DMH.

Observation of Tumor Development

The study was terminated 280 days (40 weeks) after the first injection of DMH. The rats were sacrificed at the time of termination, or when they became moribund. At autopsy, the entire intestinal tract was opened and examined for microscopic alterations. Viscera were screened for metastases. Tumors suspected areas were processed for histologic examination.

RESULTS

In Table 1, the results of various schedules of OK-432 administration on DMH-induced intestinal tumor are given. Tumors were found in almost all the DMH-treated rats, regardless the administration of OK-432. There was no significant difference in the incidence of intestinal tumors or the total number of tumors per rat between the OK-432 treated and the untreated animals. Also the incidence of cancers in the developed tumors was almost the same in all groups. Concomitant experiments revealed that administration of Ftorafur or the syngeneic tumor extract had no influence on tumor occurrence. Locations of the developed tumors are shown in Table 2. Tumors occurred frequently at the proximal colon and less frequently at the small intestine, both in the OK-432 treated and the untreated groups. As shown in Table 3, extra-intestinal tumors were found less frequently in the OK-432 treated animals (6/53 in Experiment I, 1/11 in Experiment II) than in the untreated controls (7/25 in Experiment I, 4/10 in Experiment II); this difference was not statistically significant. The two groups did not differ for distribution of the extra-intestinal lesions. Mean survival times and survivors of each group are given in Table 4, in which the animals remained alive at the termination of the experiment were calculated as 280-day survivors. No prolongation of life span was observed regardless the OK-432 administration.

Table 1. Influence of OK-432 administration on DMH-induced rat intestinal cancer

	No. of rats	No. of tumor bearers(%)	No. of cancer bearers(%)	No. of bowel tumor	No. of bowel tumor/rat
Experiment I					
OK-432 treated					
Group 1:0.2KE x 76* 0**, 38 wks.***	8	8	7	20	2.50
Group 2:1KE x 8 2, 4 wks.	9	9	8	24	2.67
Group 3:1KE x 8 4, 4 wks.	15	15	15	45	3.00
Group 4:1KE x 8 6, 4 wks.	10	9	8	27	2.70
Group 5:1KE x 8 8, 4 wks.	11	11	9	27	2.45
Total	53	52(98)	47(89)	143	2.70
Untreated	25	24(96)	22(88)	73	2.92
Experiment II					
OK-432 treated					
25KE x 13* 4** 13 wks***	11	10	9	24	2.18
Ftorafur	11	8	6	30	2.73
Tumor extract	5	5	5	24	4.80
Untreated	10	9	9	21	2.10

* number of times, ** starting month of drug administration, *** duration of drug administration.

Table 2. Location and number of tumor

	Jejunum & ileum(%)*	Proximal colon(%)*	Distal colon & rectum(%)*
Experiment I			
OK-432 treated			
Group 1	2	14	4
Group 2	0	22	2
Group 3	0	41	4
Group 4	0	25	2
Group 5	1	22	4
Total	3(2)	124(87)	16(11)
Untreated	1(2)	55(75)	17(23)
Experiment II			
OK-432 treated	1(5)	11(52)	9(43)
Ftorafur	7	12	11
Tumor extract	1	17	6
Untreated	1(5)	11(52)	9(43)

* (%): number of regional bowel tumors/total number of bowel tumors.

Table 3. Location and number of extra-bowel tumors

	No. of rats with extra-bowel tumor (%)*	Total no. of extra-bowel tumor	No. of extra-bowel tumor						
			skin	lung	liver	greater omentum	mesen -terium	ear duct	ascites
Experiment I									
OK-432 treated	6(11)	7	0	0	0	1	3	3	0
Untreated	7(28)	12	0	0	3	1	6	1	1
Experiment II									
OK-432 treated	1 (4)	2	0	1	0	0	1	0	0
Ftorafur	1	1	0	0	0	0	1	0	0
Tumor extract	2	4	0	0	0	1	2	0	1
Untreated	4(40)	7	1	1	0	1	2	0	2

*(%): number of rats with extra-bowel tumor/number of rats.

Table 4. Effect of OK-432 on survival time of DMH-induced intestinal cancer

	No. of rats	Survival days (mean+SD)	Survivors at day 280
OK-432 treated			
Group 1	8	276.75 + 8.60	7/8
Group 2	9	276.29 + 9.10	6/9
Group 3	15	271.14 + 14.26	10/15
Group 4	10	268.25 + 22.90	6/10
Group 5	11	277.64 + 7.47	10/11
Total			39/53(74%)
Untreated	25	267.43 + 27.81	17/25(68%)

DISCUSSION

Attempts on immunoprophylaxis and/or immunotherapy of experimental colorectal cancer using BCG have been reported with results ranging from major tumor enhancement[5] to inhibition of cancer growth.[6] Rogers and Gildin[7] and Martin *et al.*[8] treated chemically-induced intestinal carcinoma with BCG. BCG did not alter significantly the survival time or incidence of the intestinal tumors, but disseminated peritoneal metastases were more frequent in the BCG treated group. As to OK-432, Aoki *et al.*[9] reported that spontaneous leukemias in AKR mice occurred later and fewer in the group treated with OK-432.

The aim of the present experiment was directed to the following two targets; one to evaluate the inhibitory effects of OK-432 on carcinogenesis, the other to elucidate the suppressive effects of this agent on tumor growth. In experiment I OK-432 was administered throughout the course of tumor induction at 5 different times. The first appearance of bowel tumor was noticed on 137th day after the first injection of DMH, therefore, the rats of Group 1 were exposed to OK-432 at the onset of carcinogenesis. The rats of Groups 2 and 3 were attacked by OK-432 at the earlier stages of cancer. In Groups 4 and 5, OK-432 was given when the tumors became apparent and progressive. However, the results did not demonstrate any effect of OK-432 on every stage of DMH-induced intestinal carcinoma. Larger dose of OK-432 was administered in Experiment II, however, the results were also negative. Thus, the suppression of the development and growth of chemically-induced tumors seem to be difficult by such an immunopotentiator as OK-432. The

incidence of extra-intestinal tumors was low and few metastases were observed in the OK-432 treated rats, which may suggest host-mediated anticancer activity of OK-432.

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