EFFECTS OF BIOLOGICAL RESPONSE MODIFIER (OK-432) ON THE TUMOR-INDUCED METABOLIC ALTERATIONS -FAT METABOLISM-

Y. Noguchi*, A. Tsuburaya*, T. Makino*, K. Fukuzawa*, K. Nomura*, T. Yoshikawa*, A. Matsumoto*, Y. Moriya*, AND K. Masuda**

* First Department of Surgery, Yokohama City University School of Medicine, ** Central Research Laboratories, Chugai Pharmaceutical Company

INTRODUCTION

As mediators of tumor-induced metabolic alterations monokines have been suggested to play an important role in causing some of these alterations. A streptococcus pyogenes, OK-432, is a potent immunomodulator, whose effects on host metabolism in the tumor-bearing state have not been studied so far. If it can prolong the life of terminally ill patients, it should be able to alternate some of the tumor-induced metabolic alterations, which lead them to cancer cachexia. Furthermore OK-432 has been reported to induce production of a variety of cytokine like activities, interleukin 1 and 2 (IL-1 and 2), natural killer factor and tumor necrosis factor (TNF). On the contrary IL-1, IL-6 and TNF have been reported to be possible mediators of cancer cachexia by other investigators.

This study was conducted to evaluate the effects of this immunomodulator, OK-432, on the fat metabolism in the tumor-bearing state.

MATERIALS AND METHODS

Animals and treatment

Twenty-six male Fischer 344 rats (Japan Charles River Laboratory), weighing 150-200 gm were used. One group of rats

were injected with 10 million viable (by trypan blue exclusion method) methylcholanthrene induced sarcoma (MCA sarcoma) cells subfascially in the left flank (TBR). The other group had injection of 1 ml of Hanks saline solution (CTR).

This tumor is characterized by rapid growth, local invasiveness and minimal metastatic potential. Histologically the tumor cells are similar to those of rhabdomyosarcoma. Food intake of tumor-bearing animals were constant until 19 days after tumor inoculation, when it decreased 40%. They died of cachexia in 29±2 days after inoculation¹⁾.

These rats were divided into 4 groups ; TBR with OK-432 injections (TBR/OK), CTR with OK-432 injections (CTR/OK), TBR with normal saline injections (TBR/NS) and CTR with normal saline injections (CTR/NS). Two KE of OK-432 were injected subcutaneously every other day from the 11th day after tumor inoculation for 5 times. They were housed in a light and temperature controlled room and were studied on the 21 day after inoculation in the 24 hour fasted state. After ether anesthetics, blood was drawn by a aortic puncture, liver and epididymal fat pad (EFP) tissues were

and freeze-clamped in liquid nitrogen at -70°C until assayed.

Glucose and TG measurements

Glucose was measured by an enzymatic method. TG levels were measured by a modification of the procedure of Bucolo and David.

Lipoprotein lipase activity measurement

Epididymal fat pad (EFP), approximately 50 mg, were homogenized in 0.3 ml of extraction buffer, containing 0.2M TrisHCl, 0.73% sucrose, 1% fatty acid-free albumin, 10 units/ml heparin, 0.5% deoxycholate and 0.02% Nonident P-40, pH 8.5. The homogenate was spun at 12,000 rpm at 4°C in Eppendorf centrifuge for 10 minutes. An infranant below fat cake was saved for determination of LPL activity according to the method of Nilsson-Ehle and Schotz²¹. The amount of $[9,10-^3\text{H}]$ oleic acid released was calculated after one hour of incubation at 37°C .

Malic enzyme measurements

Livers were homogenized in three volumes of cold 0.25M sucrose. A cytosol fraction was obtained by centrifugation in 40,000 rpm (Beckman) for 45 minutes. Malic enzyme activity was measured by procedure of Hsu and Lardy. Controls in which malate were omitted from the assay reaction were run for all samples, and values obtained were subtracted as background.

Protein was measured by a Bradford's assay.

Statistic significance

Statistic significance was analyzed by Wilcoxon test, one way analysis of variance (ANOVA) and Dunnett's multiple comparison test for the data of more than three groups.

RESULTS

The steady increase of the weights of TBR over controls

were mere reflection of an increasing tumor size. Expected carcass weight derived from the formula started to decrease from 7th post inoculation day. There were no difference in food intake between four groups during this experimental period. Although there were significant decrease in EFP weight and increase in liver weight among TRB, compared with control groups, no difference was appreciated between TBR/OK and TBR/NS.

Hypoglycemia, hypertriglyceridemia and elevated cortisol levels were observed in the tumor-bearers over controls, however, the difference, depending on the injections of OK-432 were not significant.

LPL activities were significantly decreased in tumor bearers than controls; 9.99 ± 3.33 cpm/whole tissue in TBR/OK, 6.18 ± 3.42 in TBR/NS, 17.54 ± 4.00 in CTR/OK, and 22.89 ± 8.94 in CTR/NS, respectively. The difference between TBR/OK and TBR/NS was not significant.

Malic enzyme activities were not significantly different among 4 groups by ANOVA ($p=0.1773$), although ME of tumor bearers had a trend to decrease, compared with that of control rats, 1.26 ± 0.64 u/g prot for TBR/OK, 1.67 ± 1.33 for TBR/NS, 2.52 ± 1.06 for CTR/OK and 1.94 ± 0.62 for CTR/NS.

1. Popp MB, Morrison M, Brennan MF. Total parenteral nutrition in a methylcholanthrene-induced rat sarcoma model. Cancer Treat Rep 65(suppl 5): 137-43, 1981.
2. Nilsson-Ehle P, Garfunkel AS, Schotz MC. Lipolytic enzyme and plasma lipoprotein lipase metabolism. Ann Rev Biochem 49:668-693, 1980.

Key Words: lipoprotein lipase, malic enzyme