THE MECHANISM OF ANTITUMOR ACTIVITY OF TOTAL GLUCOSIDES EXTRACTED FROM CYNANCHUM AURICULA-TUM ROYLE (CA)

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Since the chemical structure of total glucosides from Cynanchum Auriculatum Royle (CA) is similar to that of the steroline of Marsdenia Condurago Reich, a compound which exhibits antitumor activity, research into the antitumor activity of CA was carried out. Its mechanism of action was studied in vivo with C57BL/6 mice bearing Lewis lung carcinoma and in vitro, with two mouse tumor cell lines: S180 and EAC. CA inhibited to a certain extent the growth of subcutaneously inoculated Lewis lung carcinoma and its pulmonary metastasis, and augmented the antitumor effect of cyclophosphamide. It showed a killing effect on the EAC and S180 tumor cells of mice in vitro as well. It blocked the tumor cells of solid Lewis lung carcinoma from entering into the S stage from G1 and inhibited DNA synthesis of S180 and EAC tumor cells of mice in vitro. It also markedly increased the number of mononuclear Mφ of tumor bearing mice, stimulated the macrophagic activity of their intraperitoneal Mφ, raised the percentage of ANAE(+) lymphocytes in peripheral blood and enhanced the ABC reaction and antibody formation in tumor bearing mice.

Cynanchum Auriculatum Royle is one of the chief varieties of the tonic Cynanchum bungei Dcne. Since the chemical structure of the total glucosides extracted from Cynanchum Auriculatum Royle (CA) is similar to that of the steroline of Marsdenia Condurago Reich, which has exhibited antitumor activity, we studied the antitumor activity of CA and its mechanism of action in vivo and in vitro.

MATERIALS AND METHODS

Drugs

CA was supplied by Professor Gong Shusheng who extracted it from Cynanchum Auriculatum Royle planted in Binhai county, Jiangsu Province. Cyclophosphamide (CY) was produced by the Shanghai No. 12 Pharmaceutical Works.

Major Reagents

Propidium iodide (PI) and RNase were purchased from Sigma. 3HTdR was obtained from the Institute of Nuclear Energy, Beijing. RPMI-1640 was imported from Serva corporation.

Animals

Male C57BL/6 mice weighing 20-22 grams, were used. The animals were divided randomly into four groups, each including 12 mice. Group 1 received distilled water (DW) as a control once daily for 10 days. In group 2, CA was injected intraperitoneally at 225 mg/kg daily for 10 days (CA group). The third group (CY) received cyclophosphamide intraperitoneally only twice at 40 mg/kg each time. Mice in group 4 (CA + CY) were given CA at 150 mg/kg daily for 10 days, and CY twice at 40 mg/kg each time.
Inoculation of Tumor

The tumor cell suspension was prepared under sterilized conditions from subcutaneous Lewis lung carcinoma of C57BL/6 mice inoculated 12 days before. The suspension was diluted with normal saline and contained $1.5 \times 10^7$ tumor cell per milliliter. Each mouse received 0.2 ml of the tumor cell suspension ($3 \times 10^6$ cells) injected subcutaneously into the right axillary region.

Determination of the Weight of the Tumor and Its Pulmonary Metastasis

Drug administration began on the second day after tumor implantation. The mice were sacrificed 12 days later and autopsied. Subcutaneous tumor mass, lungs, thymus and spleen were taken out and weighed and the tissue sections were routinely prepared. Pulmonary metastatic foci were detected with a light microscope.

The DNA Relative Content and Cell Cycle as Detected by FCM

A piece of fresh tumor tissue was taken from each mouse as soon as they were autopsied and a single cell suspension was prepared carefully. After digestion with RNase at 37°C in the water bath, it was stained with Propidium iodide (PI). The DNA relative content was detected by FACS-420 flow cytometry.

Growth Rate of Tumor Cells In Vitro

Ascitic tumor cells of S180 and Erich carcinoma (EAC), obtained from Kunming mice after five days of inoculation were used. The tumor cells were cultured with RPMI-1640 medium in plastic microtiter plates and adjusted to $2 \times 10^6$ cells/ml. To each well a 0.1 ml cell suspension ($2 \times 10^5$ cells) and 0.1 ml of CA at 0.25-4.00 mg/ml were added. In the control well an equal volume of culture medium was used instead of CA. After 24 hours, the tumor cells were stained with trypan blue and the vital cells counted. At the same time, smears were made and stained with Giemsa to observe the morphological changes in the tumor cells.

Assay of DNA Synthesis with $^{3}$HTdR Incorporated into Tumor Cells

After 20 hours of culture in a medium with CA, 0.2 μCi $^{3}$HTdR was added to each well and the tumor cells were cultured for six more hours. Then the tumor cells in each well were harvested to determine the cpm with the Beckman Liquid scintillation counter.

Determination of Immunological Parameters

Before the mice were killed, their blood was collected from the retro-ocular sinus in order to count the total number of white blood cells and to differentiate them. The percentage and index of nitraperoxideal macrophages were determined using the method of "climbing slides" while ANAE labed lymphocytes were used to differentiate the lymphocyte subpopulation. Antigen binding cells (ABC) were detected by the specific rosette formation test. The humoral immunity was determined by using the plaque forming cells test.

RESULTS

Inhibitory Effect of CA on Subcutaneous Lewis Lung Carcinoma and Its Pulmonary Metastasis in C57BL/6 Mice

A small tumorous nodule could be palpated from the 5th day after transplantation of Lewis lung carcinoma in the control mice. It could not be detected till the 6th day in the mice of CA group, however, and the tumors in this group grew more slowly than those in the control group. Tumorous nodules in the mice in the CY and CA + CY groups appeared even later. They grew even slower, especially in the CA+CY group. The mean tumor weights in the CA, CY and CA + CY groups were lighter than those in the control group. The differences were statistically significant. There were also marked tumor weight differences between the CA + CY group and the CA group or the CY group (Table 1). The results of histological examination of lung tissue indicated that the number of metastatic foci and tumorous emboli in the mice of the CA group was markedly less.