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A Study on the Anticancer Activity of Propolis

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Abstract

This study was designed to observe the anticancer activity of propolis on human rectal (HRT-18) and human colon (HCT-48) cancer cell lines *in vitro*, and on sarcoma-180 cells *in vivo*. The proliferation of HRT-18 and HCT-48 cancer cell lines was potently inhibited in proportion to the concentration of propolis. The survival time of the mice inoculated with sarcoma-180 cells was increased modestly by the administration of propolis compared to the control. Those observations suggest that propolis has anticancer effects against some of the cancer cell lines *in vitro* and *in vivo*.

Key words: propolis, anticancer activity, HRT-18, HCT-48, sarcoma-180

INTRODUCTION

A medical cure for cancer is one of the most difficult problems modern medical science faces. Most of the anticancer reagents used recently in the clinical field are chemically synthetic compounds. Repeated use of these compounds has led to the development of resistance to the agents in the tumor cell lines (1) and adverse effects on human health such as alopecia, leucopenia, sterility and secondary malignancies in clinical trials (2). Therefore, several attempts (3-5) have been made to develop new anticancer and cancer prophylactic agents from naturally occurring compounds, which have fewer side effects. It has been reported that the extracts of several natural products had cytotoxic activities against certain cancer cells both *in vitro* and *in vivo* (6-9).

Propolis, a folk medicine employed in treating various ailments, is a resinous material gathered by honey bees from the buds and bark of certain trees and plants, and stored inside their hives. Many important pharmaceutical properties have been ascribed to propolis, including anti-inflammatory, antiviral, immunostimulatory and carcinostatic activities (10-13). Anti-tumor activity of propolis against mouse cancer cells has been reported (14-18).

In this study, we assessed the *in vitro* cytotoxic activity of propolis against human rectal (HRT-18) and human colon (HCT-48) cancer cell lines and observed the effect of propolis on the survival time of sarcoma-180 tumor-bearing mice.

MATERIALS AND METHODS

Materials

Propolis was obtained from Beehive Botanicals Inc (Hayward, WI). ICR mice were purchased from Korea Experimental Animals Ltd (ChungBuk, Korea). Dulbecos Modified Eagle Medium (DMEM), horse serum, fetal bovine serum and trypsin-EDTA were obtained from Grand Island Biological Co (Grand Island, NY). All other chemicals were reagent grade.

Tumor cell lines and cancer cell culture

Cancer cell lines used in these experiments were human rectal cancer cell (HRT-18), human colon cancer cell (HCT-48) and murine ascitic sarcoma-180. HRT-18 and HCT-48 were respectively cultured by the procedure of Hwang et al. (7). Cancer cells were grown in DMEM containing 5% fetal bovine serum, penicillin (100 units/ml) and streptomycin (10 μg/ ml) in T-75 flask or 35 mm petri-dish at 37°C under 5% CO_2 tension. Sarcoma-180 cells were maintained in ICR Swiss mice by transplantation with intraperitoneal injection of the cells of mice every 10 days.

Preparation of propolis

Propolis was dissolved in ethanol, diluted in distilled water, filtered through sterilized Milipore filter disc (0.22 μm, Bedford, Mass.) and mixed with cancer cell liquid media. The final concentration of ethanol was less than 0.02%.

Measurement of inhibitory effects of propolis on growth of human cancer cells

HRT-18 and HCT-48 cells cultured in a T-75 flask were treated with trypsin, isolated and diluted with liquid media. Cells were distributed with the volume of 3 ml to a set of 35 mm petridish and cultured for 24~48 hours. When the number of cells reached about $1\sim2\times10^5$ cells/dish (HRT-18) and $4\sim5\times10^4$ cells/dish (HCT-48), the liquid media were replaced by fresh liquid media containing propolis at indicated concentrations. To measure the growth curve, propolis-

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treated and control cells were treated with trypsin, washed with 0.9% saline and counted every 24 hours by the Coulter counter (model ZBI, Beds, England). In the control group, distilled water containing the same amount of ethanol without propolis was treated instead. On the basis of the number of cells of the control group, the number of cells of the experimental group was compared to each other. All tests were triplicated. The proliferation rate and death rate were calculated as follows:

$$\text{Proliferation rate (\%)} = \frac{\text{Cell number of experimental group at each culture time - Cell number at zero time}}{\text{Cell number of control group at each culture time - Cell number at zero time}} \times 100$$

$$\text{Death rate (\%)} = \frac{\text{Cell number of experimental group at each culture time - Cell number at zero time}}{\text{Cell number at zero time}} \times 100$$

Cell size distribution

Size distribution curves of HRT-18 treated with two concentrations (30 and 50 μg/ml, respectively) of propolis were determined in comparison with those of the control cells after 24 hours of incubation by using the analyzer at Manometer count mode setting.

Effect of propolis on survival time of tumor-bearing mice

Twenty ICR mice (body weight, 20-25 g) were divided into two groups (each group contained 10 mice). All mice were intraperitoneally injected with 0.1 ml (1.0×10^6 cells/mouse) of sarcoma-180 cells on day 0, and survived mice were counted daily. Experimental groups were intraperitoneally injected everyday with 0.1 ml/mouse of propolis (20 mg/ml) for 15 days from day 1. Mice of the control group were administered with saline (0.1 ml/mouse) containing same concentration of ethanol except propolis during the same period. On the 7th and 14th day after injection of sarcoma-180 cells, the body weight of all mice was measured and the mean survival day was calculated from day 0 to day 30 (19). The prolongation ratio (%) was calculated as follows:

$$\frac{\text{Mean survival day of experimental group - Mean survival day of control group}}{\text{Mean survival day of control group}} \times 100$$

RESULTS AND DISCUSSION

Inhibitory effects of propolis on growth of human cancer cells

As shown in Fig. 1, inhibitory effects of propolis on the growth rate of HRT-18 cells *in vitro* was measured. There were no distinctive differences in growth pattern between the control group and the propolis-treated group (10 μg/ml).

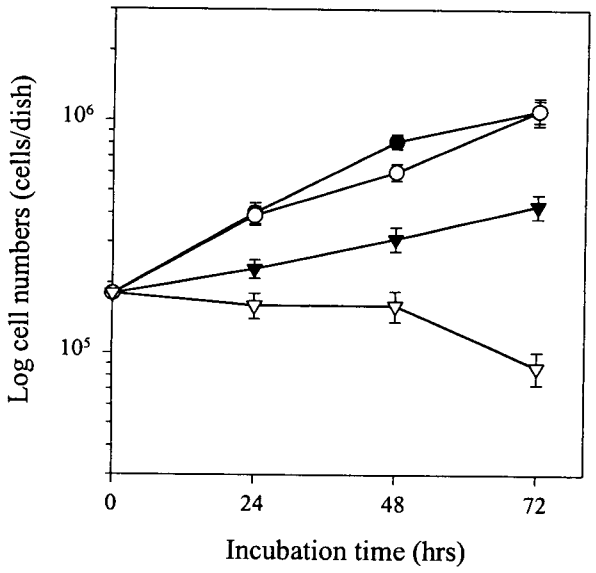

Fig. 1. Growth curves of HRT-18 cells in the culture medium containing propolis. Data were presented as means ± SD (n=3). Closed circle, control group without the addition of propolis; Open circle, treated group with 10 μg/ml of propolis; Closed triangle, treated with 30 μg/ml of propolis; Open triangle, treated with 50 μg/ml of propolis.

However, the proliferation of HRT-18 cells was inhibited by the treatment of propolis at a concentration of 30 μg/ml to the extent of 72% and 79% after 24 and 72 hours, respectively. The proliferation rates were 22.7%, 20.6% and 27.2% after incubation of 24, 48 and 72 hours, respectively. Especially in the experimental group treated with 50 μg/ml of propolis, the proliferation was markedly reduced and cells were dead. The number of cells was decreased compared to the initial cell number. Death rates were -11.1%, -11.1% and -51.2% after incubation of 24, 48 and 72 hours, respectively.

HCT-48 cells, in the control group and in experimental groups containing 10, 30 and 50 μg/ml of propolis, were respectively cultured for 72 hours, and growth curves were illustrated in Fig. 2. The growth inhibition induced by 10 μg/ml of propolis against HCT-48 cells was not significant during the incubation period, but significantly potent inhibition was found in experimental groups treated with 30 and 50 μg/ml of propolis. When HCT-48 cells were cultured in the medium containing 30 and 50 μg/ml of propolis for 72 hours, cell death occurred and cell number remarkably decreased to 87% and 98% compared to the initial cell number, respectively. Death rates at a concentration of 30 μg/ml were -23.4%, -75.0% and -87.2% after incubation of 24, 48 and 72 hours, respectively. And at a concentration of 50 μg/ml, -89.4%, -97.8% and -97.8%, respectively.

These results show that propolis potently inhibited the proliferation of some human cancer cell lines *in vitro*. The types of cytotoxic effect by all of the anticancer reagents are as follows: first, only dose-dependent, second, only time-dependent, third, simultaneously dose- and time-dependent (20). From

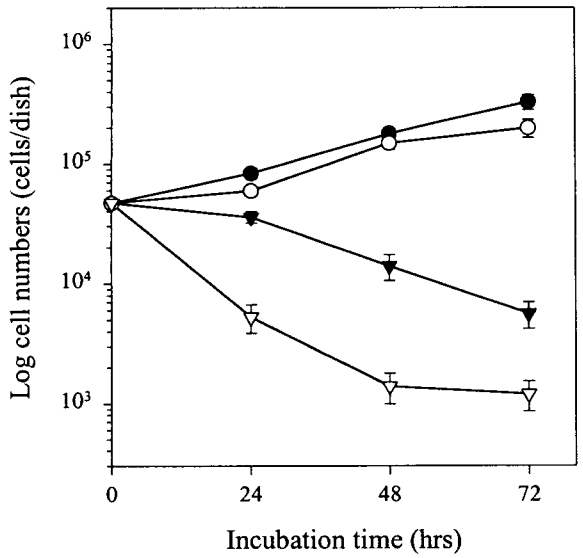

Fig. 2. Growth curves of HCT-48 cells in the culture medium containing propolis. Data were presented as means±SD (n=3). Closed circle, control group without the addition of propolis; Open circle, treated group with 10 μg/ml of propolis; Closed triangle, treated with 30 μg/ml of propolis; Open triangle, treated with 50 μg/ml of propolis.

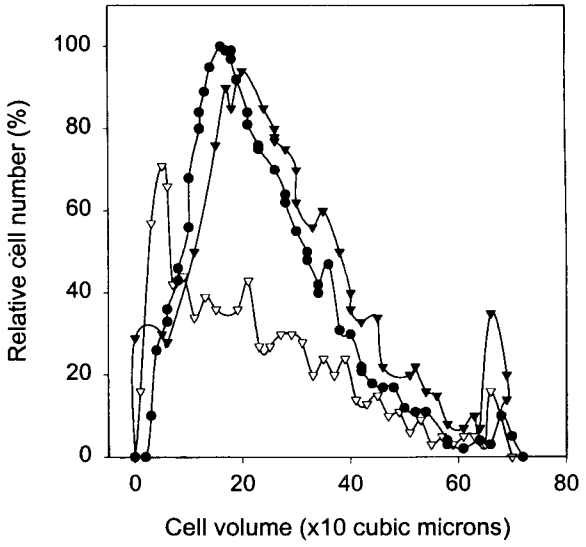

Fig. 3. Size distribution curves of HCT-48 incubated with and without propolis for 24 hours. Closed circle, control group without the addition of propolis; Closed triangle, treated with 30 μg/ml of propolis; Open triangle, treated with 50 μg/ml of propolis.

the above results, it is considered that the effects of propolis are simultaneously dose- and time-dependent. Therefore, if dose and time are well controlled *in vitro*, it is expected that propolis is a successful medicine to inhibit the growth of human cancer cells or to kill them. Based on the above experimental results, the cytotoxic effect of propolis was determined to be efficacious to certain cancer cells, and it seems to be desirable that the detailed biochemical mechanism of cytotoxic action be investigated further.

Influence of propolis on the cell size distribution

Fig. 3 showed that the changes of the cell size by the action of propolis seems to be related to the decrease of cell number during the incubation. The overall changes of size distribution of HCT-48 treated with 50 μg/ml of propolis were determined after 24 hours of incubation. The peaks of the size distribution curve at a concentration of 50 μg/ml of propolis were greatly changed to less than 40 cubic microns (c.m.) within 24 hours of incubation, while those of the control cells were maintained at 144 c.m. during incubation. It appears that the addition of propolis to the culture medium caused the decrease of cell size which was succeeded by cell death. Propolis is presumed to induce the intracellular derangement of the cancer cell metabolism which reflects the inability of cells to adapt to the metabolic effect of propolis and sustain a viable state. Detailed mechanisms regarding this phenomenon must be studied in view of cell morphology.

Effect of propolis on survival time of tumor-bearing mice

As shown in Table 1, propolis had a prolongation effect on the life-span of ICR mice inoculated with sarcoma-180 cells by 16.8%. The average life-span of the experimental group was 18.1±1.68 days and that of the control group was 15.5±1.67 days. These results showed that the life-span of the propolis-treated group has modestly been prolonged against sarcoma-180 cells. It was therefore thought that propolis might have the anticancer activity *in vivo* as well as *in vitro*.

Table 1. Effect of propolis on life-span of tumor-bearing ICR mice inoculated with sarcoma-180 cells

Group	Survival time[1)] (day)	Prolongation ratio (%)
Control	15.5±1.67	0
Treatment (2 mg/mouse)	18.1±1.68*	16.8

[1)]All values were expressed as means±standard error. *$p<0.05$

Further studies to investigate the anticancer activity of propolis against nude mice inoculated with human cancer cells *in vivo* are warranted.

In conclusion, the proliferation of human rectal (HRT-18) and human colon (HCT-48) cancer cell lines was potently inhibited *in vitro* in proportion to the concentration of propolis, respectively. The survival time of the mice inoculated with sarcoma-180 cells was modestly increased by the administration of propolis *in vivo*. Those observations suggest that propolis has anticancer effects against some of the cancer cell lines *in vitro* and *in vivo*.

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