Proceedings of the 5th International Ginseng Symposium, Seoul, 1988

A Tumor Growth Inhibitory Substance Isolated from *Panax ginseng*

Mitsuo Katano*, Hiroshi Yamamoto*, Hisashi Matsunaga**

*Department of Surgery and **Hospital Pharmacy, Saga Medical School, Nabeshima-cho, Saga 840-01, Japan

Abstract

During a series of studies aimed at isolation of the tumor growth inhibitory substance from *Panax ginseng*, we found a new type of antitumor substance. The substance was isolated from a powder of the root of *Panax ginseng* C.A. Meyer, which is commonly used for various diseases as a commercial medical drug by the name of Korean Red Ginseng Powder in Japan. Data from infrared spectra, proton and carbon-13 nuclear magnetic resonance, and high resolution mass spectra were identical with those of panaxytriol. Panaxytriol isolated from Korean Red Ginseng Powder (Nikkan Korai Ninjin Co., Ltd., Japan) inhibited the growth of several kinds of human and murine malignant cells in vitro. Although the detailed mechanism of cell growth inhibition by panaxytriol is yet to be elucidated, panaxytriol's action appeares to be more dose-dependent than time-dependent.

From ancient times, *Panax ginseng* has been used for treatment of various diseases as an important folk drug in Asian countries. Since *Panax ginseng* is now used in Japan as a commercial medical drug, it is oftenly given to patients with malignant diseases. Therefore, it is important to study what effect *Panax ginseng* has on the cell growth of malignant cells. We report here a new tumor growth inhibitory substance isolated from *Panax ginseng*.

Effect of crude extracts from *Panax ginseng* on cell growth: Tumor growth inhibitory activity was detected in crude extracts from a powder of heat-treated roots of *Panax ginseng* C.A. Meyer, which is used in Japan as a commercial medical drug by the name of Korean Red Ginseng powder (KRG powder: Nikkan Korai Ninjin Co., Ltd., Kobe, Japan). At first, KRG powder (1 g) was subjected to a 12-hours extraction at room temperature with various kinds of solvents. Each of the extracts was dissolved in 10ml of RPMI 1640 culture medium.

The tumor growth inhibitory activity of RPMI 1640 medium-soluble extracts was measured as follows. Fifty microliters of human gastric adenocarcinoma cells, MK-1 cells, adjusted to 2×10^5 cells/ml and 50μl of the extract solution were plated in microtiter wells and incubated for 48 hours at 37°C. After culture, the culture medium was gently removed: tumor cells that attached to the plate were collected by trypsinization. Dead cells were determined by trypan blue dye exclusion. Tumor growth inhibitory activity is expressed as follows:

$$\%\ \text{growth inhibitory activity or}\ \%\ \text{growth inhibition} = \left(1 - \frac{\text{no. of viable cells in extract containing RPMI medium}}{\text{no. of viable cells in RPMI medium}}\right)\times 100$$

As shown in Figure 1, tumor growth inhibitory activity was detected in both water-and organic solvent-extracts. This result indicates that KRG powder-derived tumor growth inhibitory substance(s) possesses an unusual property of being soluble in both water and organic solvents.

Isolation and purification of a tumor growth inhibitory substance from KRG powder: KRG powder (200 g) was extracted with ethyacetate (AcOEt) at room temperature for 12 hours. AcOEt-extracts were applied to a column chromatography using silica gel and fractionated with AcOEt-hexane (1:1). Each fraction was assayed for tumor growth inhibitory activity against human gastric adenocarcinoma MK-1 cells. Positive fractions were collected and purified gradually by preparative TLC(silica gel) using AcOEt-hexane(1:1) and chloroform-methanol (99:1) as a developer to obtain colorless oil(34 mg) as shown in Figure 2. The purity of colorless oil was confirmed by two-dimensional TLC. The yield of a tumor growth inhibitory substance was about 0.017% in this separation procedure. Finally the colorless oil were crystalized from distilled water.

Chemical structure of a tumor growth inhibitory substance: Infrared absorption spectra were determind on a Hitachi 270-30 spectrophotometer. Proton and

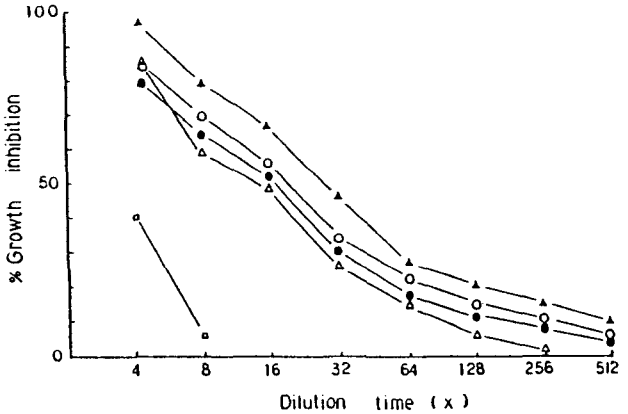

Figure 1. Tumor growth inhibitory substance in extracts from KRG powder. KRG powder (1 g) was extracted with various kinds of solvents. Extract were dissolved in RPMI 1640 medium and assayed for tumor growth inhibitory activity using MK-1 human gastric adenocarcinoma cells as a target. ▲, Methanol; ○, water; ●, Ethyl acetate; △, Butanol; □, Hexane.

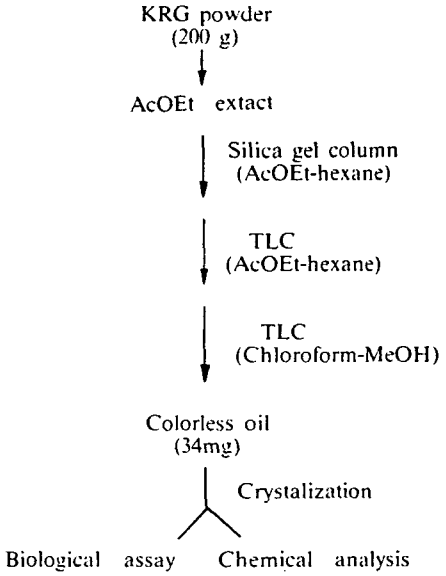

Figure 2. Isolation of a tumor growth inhibitiory substance from KRG powder.

carbon-13 nuclear magnetic resonance spectra were determined on a JMS-GSX 400 in deuteriocholroform solution with tetrametylsilane as internal standard. Mass spectra were recorded with a JMS-D 300 spectrometer by the electron impact (EI) and chemical ionization (CI) methods.

All chemical analysis were performed using the final product. Data from these experimental analysis were identified with those of panaxytriol. heptadeca-1-ene 4. 6-diyne-3.9.10-triol(1.2). Namely. one of tumor growth inhibitory substance(s) extracted from KRG powder is panaxytriol. Figure 3 shows the molecular structure of panaxytriol.

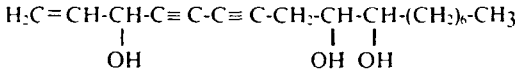

Figure 3. Molecular structure of panaxytriol.

Effect of panaxytriol on the growth of varying cultured cells : The effect of panaxytriol on cell growth was examined *in vitro* using various kinds of cultured cells. For the assay. pure panaxytriol isolated from KRG powder was used. MK-1 human gastric adenocarcinoma cells. M14 human melanoma cells. SW620 human colonic cells. Hela human uterus adenocarcinoma cells. K562 human erythroleukemic cells. B16 mouse melanoma cells. and MRC-5 human embryo-derived fibroblasts were used as target cells. Panaxytriol inhibited the growth of various kinds of human cultured tumor cell lines in a dose-dependent fashion. Table 1 shows the concentration of panaxytriol required to obtain 50% growth inhibition (ED50). Although the growth of human MRC-5 fibroblasts was also inhibited by panaxytriol. ED50 was over 40 μg/ml.

In order to examine the process of growth inhibition by panaxytriol. time-course study was performed using MK-1 cells as a target. As shown in Figure 4. panaxytriol inhibited the cell growth in a dose-dependent fashion between 2.5 and 0.08μg/ml.

Table 1. Effect of panaxytriol on the growth of varying culture cells.

Cell line[a]	ED50 (μg/ml)[b]
MK-1	0.6
B16	1.6
SW620	2.0
M14	2.5
HeLa	10.0
K562	10.0
MRC-5	>40.0

[a] MK-1. human gastric adenocarcinoma : B16. mouse melanoma : SW620. human colonic adenocarcinoma : M14. human malignat melanoma : HeLa. human uterus adenocarcinoma : K562. human erythroleukemia : MRC-5. human fibroblast.

[b] ED50 shows the concentration of panaxytriol required to obtain 50% growth inhibition.

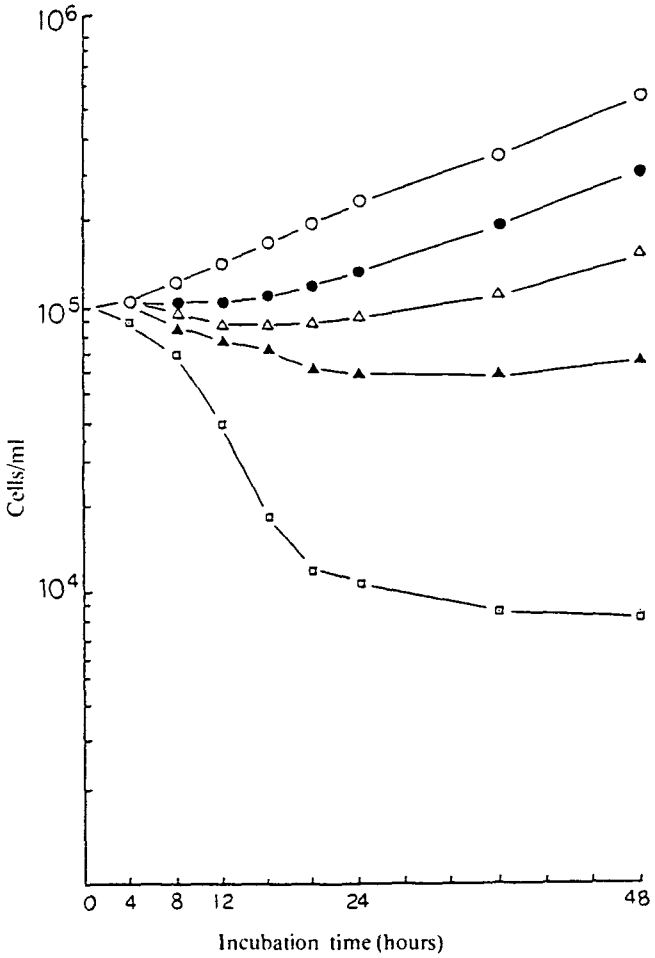

Figure 4. Effect of panaxytriol on growth of MK-1 human gastric adenocarcinoma cells. MK-1 cells were cultured with varying concentrations of panaxytriol. ○. 0 μg/ml : ●. 0.3 μg/ml : △. 0.6 μg/ml : ▲. 2.5 μg/ml : □. 5μg/ml.

However. the growth rate almost recovered that control MK-1 cells after some culture intervals. The higher is concentration of panaxytriol. the more the recovery of cell growth is delayed. On the other hand.

panaxytriol causes a marked cell destruction at concentrations over 5 μg/ml at early stage of culture. However, panaxytriol dose not cause such a cell destruction against human peripheral red blood cells and human peripheral lymphocytes even at concentrations over 40 μg/ml. These findings indicate that panaxytriol acts as cytotoxic substance at high concentrations, and that it acts as cytostatic substance at low concentrations. Differing from inhibitors of DNA synthesis such as mitomycin C, cytostatic action by panaxytriol is reversible.

Cell-free supernatants were obtained at varying intervals from the mixed culture of panaxytriol and MK-1 cells and added to newly prepared MK-1 cells for examination of the remaining inhibitory activity in the culture supernatants. As shown in Figure 5, tumor growth inhibitory activity in the supernatants rapidly decreased during cultivation. This phenomenon indicates that panaxytriol is consumed by MK-1 cells at an early stage during cultivation, and that early recovery from the growth inhibition is partly due to rapid consumption of panaxytriol by target tumor cells. Rapid consumption of panaxytriol by target cells also suggests that the inhibition dose not necessarily require continuous contact between panaxytriol and target cells throughout the culture. In fact, the cell growth was significantly inhibited by a transient contact, i.e. 60 minutes, with panaxytriol at concentrations over 2.5 μg/ml. Although there is a tendency that the consumption by sensitive cells is larger than that by resistant cells, it is under investigation if the quantity of consumption is directly related to the sensitivity against panaxytriol.

Panaxytriol's action seems to be more dose-dependent than time-dependent. Part of the interim results indicates that panaxydol (3), a similar acetylenic alcohol, also exhibits growth inhibitory activity against tumor cells.

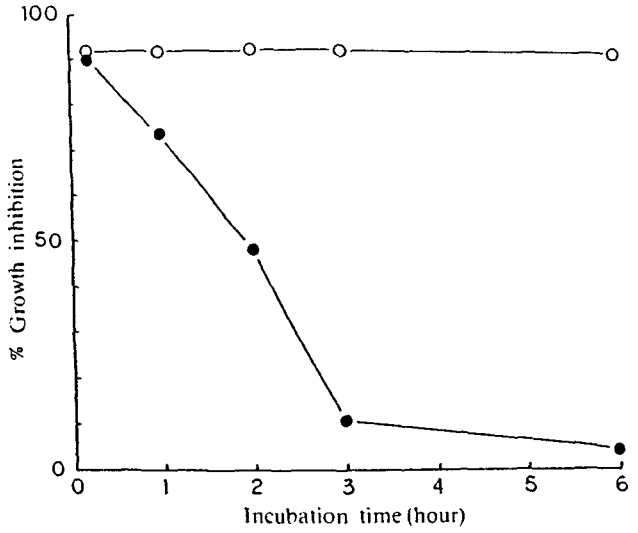

Figure 5. Consumption of panaxytriol by MK-1 cells. MK-1 cells (10^6 cells) were incubated with 1 ml of panaxytriol-containing medium (5μg/ml). Cell-free supernatants were obtained at varying intervals from the culture and added to newly prepared MK-1 cells for examination of the remaining inhibitory activity in the culture supernatants. ○, panaxytriol-containing medium only; ●, panaxytriol-containing medium with MK-1 cells.

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고려홍삼분말중의 항종양 활성물질

Mitsuo Katano*, Hiroshi Yamamoto* and Hisashi Matsunaga**

*Department of Surgery and **Hospital Pharmacy
Saga Medical School, Saga, Japan

우리는 경험적으로 인삼이 훌륭한 치료효과로서 항종양효과가 있다는 것을 알고 있다. 인삼으로부터 종양발육 억제효과가 있는 물질을 분리하는 과정에서 우리는 새로운 타입의 항종양 물질을 발견하였다. 이물질은 *Panax ginseng* C.A. Mayer의 분말로부터 분리하였는데 이 분말은 일본에서 홍삼분말이라는 이름으로 의약품으로서 여러가지 질병치료에 사용되고 있다. IR, ^{1}H, ^{13}C-NMR 및 MS에 의한 분석결과로 이 물질은 panaxytriol로 동정 되었다. 고려홍삼에서 분리된 panaxytriol은 *in vitro* 시험에서 몇가지의 인체암세포와 악성백혈병 세포의 성장을 억제하였다. 비록 panaxytriol에 의한 세포성장억제에 대한 상세한 작용기전은 알려져있지 않으나 panaxytriol의 효과는 처리시간보다는 농도 의존성이 있는 것으로 밝혀졌다.