Cytotoxic Activity of Polyacetylene Compounds in *Panax ginseng* C. A. Meyer

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The effects of the three polyacetylene compounds, panaxynol, panaxydol and panaxytriol, on *in vitro*-cell growth were studied. These compounds are much different in their water-solubility. In order to increase water-solubility, solid complexes of polyacetylene compounds with α-cyclodextrin (CD) were prepared. Accurate concentrations of the active compounds in a culture medium were determined by gas chromatography. All of these CD-complexes inhibited the growth of various kinds of cultured cell lines in a dose-dependent fashion. The cell growth inhibitory activity of these complexes was much stronger against malignant cells than against normal cells. A continuous contact between the compounds and target cells was not necessarily required for growth inhibition. And the inhibition was cytotoxic at high concentrations and cytostatic at low concentrations. These findings indicate that these polyacetylene compounds' mode of action is more dose-dependent than time-dependent. The panaxynol, panaxydol and panaxytriol contents in red ginseng powder were 250, 297 and 320 μg/g, respectively.

Introduction

In previous papers,[1] we reported that one of the polyacetylene compounds, panaxytriol, isolated from red ginseng, was a cytotoxic substance. It is known that red ginseng contains several kinds of polyacetylene compounds such as panaxynol and panaxydol.[2] As shown in Chart 1, these compounds are structurally different only in the C-9, 10 positions. Namely, the respective C-9, 10 positions of panaxynol, panaxydol and panaxytriol are double bond, epoxy and a glycol type. It is expected that panaxynol and panaxydol, as well as panaxytriol, inhibit tumor cell growth.

In this report, we demonstrate the *in vitro*-cell growth inhibitory activity of these polyacetylene compounds isolated from red ginseng.

Results and Discussion

Panaxytriol and panaxydol are relatively soluble in water. But panaxynol is insoluble in water. It is difficult to examine the effect of these polyacetylene compounds on *in vitro*-cell growth because of their different water-solubility. Although panaxydol can be isolated from red ginseng, it is still very difficult to get pure panaxydol. Therefore, synthesized panaxydol was used in this experiment. In order to increase their water-solubility, the solid complexes of panaxynol (PN/CD), panaxydol (PD/CD) and panaxytriol (PT/CD) with α-cyclodextrin (CD) were prepared. Each of PN/CD, PD/CD and PT/CD was dissolved in an RPMI-1640 culture medium and sonicated for 3 min. The concentrations of

active panaxynol, panaxydol and panaxytriol in a culture medium were determined by gas chromatography.[1a,3]

The effect of these polyacetylene compounds on cell growth was examined *in vitro* using various kinds of cultured cells. Nude mouse-transplantable human gastric adenocarcinoma cells (MK-1), mouse malignant melanoma cells (B-16) and mouse fibroblast-derived tumor cells (L-929) were used as malignant cells. And human embryo-derived fibroblasts (MRC-5) and mesothelial cells isolated from ascitic fluids were used as normal cells. Table I shows the effects of these polyacetylene compounds on the cell growth *in vitro*. ED_{50} indicates the concentrations of polyacetylene compounds required to obtain 50% growth inhibition of target cells. The ED_{50} of panaxynol (double bond type), panaxydol (epoxyl type) and panaxytriol (glycol type) was 0.027, 0.016 and 0.171 μg/ml against MK-1 cells, respectively.

These compounds also inhibit the cell growth of normal cells. However, the ED_{50} against normal cells such as MRC-5 and mesothelial cells was very high compared to that against malignant cells. In particular, panaxytriol did not inhibit the growth of MRC-5 and human mesothelial cells by 50% even at concentrations of over 70 μg/ml. The

CH₂=CHCH(C≡C)₂CH₂CH=CH(CH₂)₆CH₃ with positions labeled 1, 2, 3, 4–7, 8, 9, 10, 11–16, 17 and OH

panaxynol

CH₂=CHCH(C≡C)₂CH₂CH–CH(CH₂)₆CH₃ with OH and epoxide O

panaxydol

CH₂=CHCH(C≡C)₂CH₂CH–CH(CH₂)₆CH₃ with OH and OH OH

panaxytriol

Chart 1

TABLE I. Effect of Each Polyacetylene Compound on Cell Growth *in Vitro*

Cell line[a]	ED_{50} (μg/ml)[b]		
	Panaxynol	Panaxydol	Panaxytriol
MK-1	0.027 ± 0.004	0.016 ± 0.005	0.171 ± 0.033
B-16	1.23 ± 0.03	1.50 ± 0.10	2.23 ± 0.25
L-929	2.50 ± 0.28	2.60 ± 0.17	4.39 ± 0.10
MRC-5	17.10 ± 1.30	11.50 ± 0.40	> 70
Mesothelial cells	32.10 ± 0.87	16.40 ± 1.10	> 70

Fifty microliters of cell suspension (1×10^5 cells) and 50 μl of each polyacetylene compound solution were plated in flat-bottomed microtiter wells and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Percent growth inhibition

$$= \left(1 - \frac{\text{no. of viable cells in medium with polyacetylene compounds}}{\text{no. of viable cells in medium without polyacetylene compounds}}\right) \times 100.$$

a) MK-1 (human gastric adenocarcinoma); B-16 (mouse melanoma); L-929 (mouse fibroblast-derived tumor); MRC-5 (human fibroblast). b) ED_{50} is the concentration of each polyacetylene compound required to obtain 50% growth inhibition. Mean ± S.D. of three experiments.
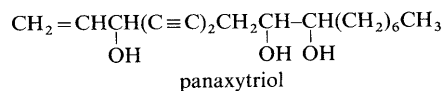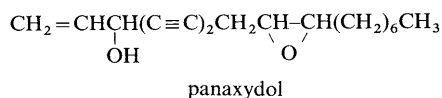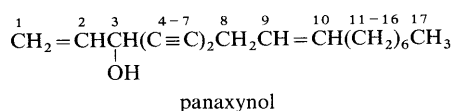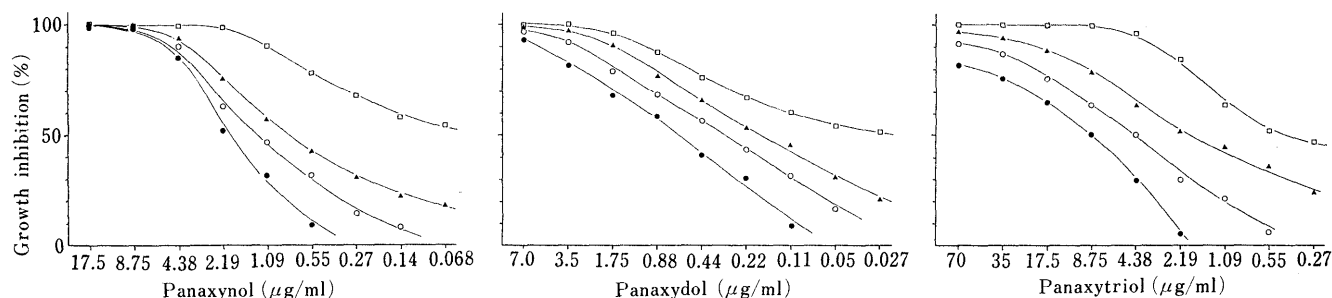
Fig. 1. Effect of Continuous Contact between Each Polyacetylene Compound and MK-1 Cells

Contact time (h): —●—, 0.5; —○—, 1.0; —▲—, 2.0; —□—, 48.

TABLE II. Concentration of Polyacetylene Compounds in Red Ginseng

Compounds	Concentration $(\mu g/g)$
Panaxynol	$250 \pm 60^{a)}$
Panaxydol	297 ± 102
Panaxytriol	320 ± 27

a) Mean $\pm$ S.D. $(n=4)$.

ED_{50} of panaxynol was 17.1 and 32.1 $\mu g/ml$ against MRC-5 and mesothelial cells, respectively. The ED_{50} of panaxydol was 11.5 and 16.4 $\mu g/ml$. Tumor specificity (ED_{50} of MRC-5/ED_{50} of MK-1) of panaxynol, panaxydol and panaxytriol was 17.1/0.027, 11.5/0.016 and more than 70/0.171, respectively. These findings indicate these polyacetylene compounds' selective actions against malignant cells.

In order to determine whether or not the growth inhibition by polyacetylene compounds required a continuous contact between the polyacetylene compounds and the target cells, MK-1 cells were cultured with each of the polyacetylene compounds for varying durations (for 30 to 120 min) at 37 °C. After the respective incubation times, the cells were washed two times with a culture medium to eliminate the polyacetylene compounds. Washed MK-1 cells were cultured in a fresh medium without the compounds for an additional 48 h. Although cell viability was not affected in this pre-treatment, cell growth was significantly inhibited even by transient contact, i.e. 30 min, with polyacetylene compounds as shown in Fig. 1. Figure 1 also indicates that the cell growth inhibition is due to the cytotoxic effects of the compounds at high concentrations, and that it is due to cytostatic effects at low concentration. The action seems to be more dose-dependent than time-dependent.

Polyacetylene compounds' contents in red ginseng were determined by gas chromatography (GC) as shown in Table II. The contents of panaxynol, panaxydol and panaxytriol were approximately 250, 297 and 320 $\mu g/g$, respectively. Recently, several investigators[4] have isolated other types of acetylene compounds such as panaxacol and acethylpanaxydol from *Panax ginseng* and the callus of *Panax ginseng*, but not from red ginseng. They also reported the compounds' *in vitro*-cell growth inhibition activity.[4b—g] We demonstrated that panaxytriol administered intramuscularly produced significant tumor growth delays in mice.[1e] These findings indicate that polyacetylene compounds may play a significant role in tumor growth, even in patients.

Conclusion

Polyacetylene compounds, *i.e.*, panaxynol, panaxydol and panaxytriol, were isolated from Korean red ginseng powder, which is used in Japan as a commercial medical drug. All of these compounds inhibited the growth of various kinds of cultured cells *in vitro* in a dose-dependent fashion. The inhibitory activity was relatively tumor selective. These results indicate that polyacetylene compounds may be a new type of cytotoxic substance.

Experimental

Infrared (IR) spectra were recorded with a Hitachi 270-30 spectrometer, ultraviolet (UV) spectra with a Shimadzu UV-240 spectrometer, proton nuclear magnetic resonance (^{1}H-NMR) spectra with a JEOL JNM-GX400 spectrometer (with tetramethylsilane as an internal standard, $CDCl_3$ solvent) and GC–mass spectrometry (GC–MS) with a Hitachi M2000. GC was done with a Hitachi 663-30 gas chromatograph. For sonication, an Astrason W-385 sonicator was used. Column chromatography was carried out on silica gel 60 (100—200 mesh, Nakarai). Thin-layer chromatography (TLC) was performed on Kiesel gel 60 plates (Merck). Spots were detected by spraying the plates with concentrated H_2SO_4 and then heating them.

Extraction and Isolation of Panaxynol Panaxynol was extracted from Korean red ginseng powder (Nikkan Korai Ninjin Co. Ltd., Kobe) and was isolated by the method used in our previous reports.[1]

Panaxynol was identified by comparison of IR, ^{1}H-NMR and GC–MS data.[2a,c]

Synthesis of Panaxydol Panaxydol was also extracted from red ginseng powder, but it was still impossible to purify. Therefore, panaxydol was synthesized from panaxynol by the method of Poplawski *et al.*[2b]

Panaxydol was identified by comparison of IR and ^{1}H-NMR data.[2b,4b]

Extraction and Isolation of Panaxytriol Panaxytriol was extracted from red ginseng and was isolated by the method used in our previous reports.[1a]

Panaxytriol was identified by comparison of IR and ^{1}H-NMR and TLC data.[1a,2a]

Preparation of Solid Complexes Each polyacetylene compound, previously dissolved in a small amount of acetone, was added to saturation with CD. Each of the mixtures were then vigorously shaken for 3 h at room temperature. The complexes, which precipitated as micro-crystalline powders, were filtered and dried under a vacuum for 24 h at 40 °C. Each complex was dissolved in an RPMI-1640 culture medium. The quantity of each polyacetylene compound in the culture medium was determined by GC.

Gas Chromatographic Conditions[1a,3] A gas chromatograph equipped with a flame-ionization detector (FID) and a moving needle solvent cut sample injector (Gasukuro Kogyo) was used. The column used was a chemical bonded fused silica capillary column coated with CP-Sil 19CB (50 m × 0.25, i.d., 0.2 μm, Gasukuro Kogyo). The injection and detector temperatures were set at 250 °C, while the column temperature was kept at 260 °C. Helium was used as a carrier gas and make-up gas at flow rates of 1.1 and 35 ml/min, respectively. The flow rates of air and hydrogen were adjusted to 400 and 40 ml/min, respectively. The split ratio was 68 : 1.

Preparation of Culture Medium for GC[1a,3] Polyacetylene compounds containing the culture medium (1.0 ml) were added to ethyl acetate (5 ml) with 50 μl (100 ng) of the internal standard, 1-docosanol. The mixture was vigorously shaken for 10 min at room temperature and centrifuged for

10 min at 1500 g. The organic phase was transferred to a glass tube and evaporated to dryness under nitrogen. The dry residue was subjected to GC.

Determination of Polyacetylene Compounds by GC The polyacetylene compounds were determined by the method used in our previous reports.[1a]

Antitumor Activity Fifty microliters of cell suspension (1×10^5 cells) on an RPMI-1640 culture medium containing 20% fetal calf serum (GIBCO Lab, N.Y., U.S.A.) and $50 \mu l$ of each of the polyacetylene compound solutions were plated in flat-bottomed microtiter wells and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air.

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