A CYTOTOXIC ACTIVITY OF EXTRACT OF *PANAX GINSENG* ROOT AGAINST SOME CANCER CELLS *IN VITRO* AND *IN VIVO*

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Abstract

This study was devised to observe the cytotoxic activity of extracts of *Panax ginseng* root against some cancer cells and to purify the crude extract.

Three kinds of cancer cells(leukemic cells L5178Y, HeLa cells and Sarcoma 180 cells) and mouse embryo cells (as normal cells) were used for this study. The ginseng roots were extracted with petroleum ether in soxhlet apparatus, and the crude extracts were purified by the silicic acid column chromatography and thin-layer chromatography methods.

The results obtained are summarized as follows;

1. Eight to ten mg of the petroleum ether extract (crude extract) were obtained from 1 g of *Panax ginseng* root, and its activities per mg were about 1,000 units.

2. Doubling time of the L5178Y cells was increased to two fold by 24 hours incubation in culture medium containing about one μg of extract per ml, and eight and ten folds higher concentration of ginseng extract were required for the Sarcoma 180 cells and HeLa cells, respectively, than for the leukemic cells(L5178Y) to inhibit the cellular growth to the same degree.

3. When the L5178Y cells were exposed to medium containing various concentration of the extract for 24 hours before initiation of the soft agar cloning procedure, about 99 % of the L5178Y cells were killed at concentration of 8 units per ml.

4. The growth rate of mouse embryo cell (as normal cell) was not affected by the culture with media containing various amounts (1.45 to 30.0 μg/ml) of the extract.

5. The crude extract could be purified about four times by silicic acid column chromatography using several solvent systems, and one spot of active compound could be obtained on the thin-layer chromatogram.

6. In the Swiss mice inoculated with Sarcoma 180 cells, a survival time of the experimental group (injection group of active compound) was extended more 1.5 to 2.0 times than the control group's(no injection group).

Introduction

Panax ginseng has been used as herbals in the Orient for several thousand years and also well known to be one of the most valued and most frequently prescribed Oriental herbals. Recently, *Panax ginseng* has been subjected to modern scientific study by many investigators.

The pharmacological activities of *Panax ginseng* can be divided briefly into next several functions, according to the reports during the past ten years: (1) the effects of *Panax ginseng* on the central nervous system (Brekhman, 1966; Lee, 1965; Oh, 1966), (2) on the resistance to various stress (Park, 1962; Kim, 1966), (3) on

growth and basal metabolism (Kim, 1970; Han, 1962), (4) on carbohydrate metabolism (Cheong, 1965; Kim, 1963), (5) on fat metabolism (Choi *et al.*, 1968; Cho & Oh, 1962), (6) on the metabolism of protein and nucleic acid (Cho 1971; Lee *et al.*, 1969) and (7) on the blood pressure and hematopoiesis (William *et al.*, 1964).

Han and his co-workers (1974) isolated two anti-inflammatory glycosides, belonging to protopanaxatriol glycosides, in the crystalline form, and designated them panax saponin A and C. Shibata (1962) designated 13 glycosides of dammarane series, and Brekhman (1962) designated 13 glycosides of dammarane series, and Brekhman (1968) reported that the basic effect in ginseng action is its capacity to increase nonspecific resistance of the organism to various untoward influences, that is adaptogenic activity, and that the effective components of ginseng are glycosides of dammarane series.

While, Oura (1972) purified a stimulating factor of protein synthesis from extract of *Panax ginseng* and designated it as prostisol, and Murata (1972) reported that the prostisol had an immunological effect against cancer by promoting the reticuloendothelial system and enhancing host resistance of cancer patients.

In view of the above reports, it should be emphasized that the pharmacological activities may be due to a group of glycosides called ginsenoside or panax saponin in the *Panax ginseng*, which is soluble in water.

In this study, it was demonstrated that a highly lipid soluble compound in the extract of Korean ginseng root has a carcinostatic activity for some cancer cells *in vitro* and *in vivo*.

Materials and Methods

Materials

Dried ginseng roots (white, six years old, a product of Kang-hwa area) were obtained from commercial sources in Seoul. Fischer's medium and Eagle medium were purchased from Grand Island Biological Co.; analytical and preparatory silica gel thin-layer chromatography sheets with fluorescent indicator from Fisher Scientific; silicic acid (Bio- Sil A, 100–200 mesh) from Bio-Rad Laboratories; millipore filter discs (Gs 0.22 μ) and accessories from Millipore Corp. from U.S.A. Horse serum, murine leukemic lymphoblasts (5187Y), murine ascitic Sarcoma cells (S-180) were a gift from Dr. Lee (Institute of Atomic Energy Research) and HeLa cell (origin of cancer in human womb), its culture medium (Eagle) and calf serum from Dr. S.B. Baek (N.I.H). Embryo cells used as normal cells were prepared from Swiss mice maintained in this laboratory.

Methods

Preparation of *Panax ginseng* extract

Panax ginseng roots were finely pulverized with a mortar, and about 3 g portions were extracted with petroleum ether for 16 hours. The crude extract was dried by evaporation with rotary evaporator under a stream of nitrogen gas, and dissolved in absolute ethanol and stored in a refrigerator.

To test the cytotoxic activities of extracts, the alcoholic solution was filtered through a millipore disc under sterile conditions and the filtrate was diluted with sterile distilled water. A small aliquot of this aqueous solution was added to the culture medium. The concentration of ethanol in the culture medium never exceeded 0.2% by volume and the inhibitory effect on the cell growth at this concentration of alcohol was negligible.

Cell culture. L5178Y cells and Sarcoma 180 cells were maintained in the Fischer's medium described by Fischer & Sartorelli(1964). HeLa and mouse embryo cells were maintained in the Eagle medium containing calf serum under similar conditions as for the culture of L5178Y cells.

Quantification of cytotoxic potency. One unit of the cytotoxic activity was arbitrarily defined as the amount of extract which in one ml of the culture medium causes a two fold increase in the apparent cellular doubling time of L5178Y cells estimated after 24 hours of incubation.

In practice, the assay was accomplished as follows; cells were grown in media containing

various amounts of the extract and the cell numbers were counted by using a Coulter counter after 24 hours of incubation.

The dose corresponding to the midpoint between the logarithm of the cell number at zero time and that of the control at 24 hours was estimated from a plot of logarithm of cell number vs. extract concentration as illustrated in Fig. 1 and Table 1.

Table 1. The unit assay of *Panax ginseng* extract on the L5178Y cells (leukemia cells)

Extract added (μg/ml)	Incubation time (hours)	
	0	24
0 (control)	3.85×10^4 cells/ml	15.8×10^4 cells/ml
0.4	//	12.9 //
0.8	//	10.1 //
1.2	//	6.0 //
1.6	//	3.5 //
2.0	//	2.8 //

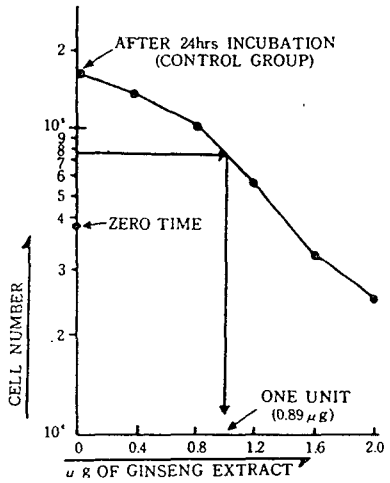

Fig. 1. Dose-response curve of the pet. ether extract of *Panax ginseng* on the growth of L5178Y cells *in vitro* after 24 hours incubation.

Silicic acid column chromatography

Fifteen grams of silicic acid in a 100 ml beaker were heated in an oven for two hours at 120° C. Chloroform (50ml) was added to the beaker, and the slurry was poured into a glass column (2 × 17 cm) with a fritted glass disc and a Teflon stopcock. The column was washed first with 50 ml of chloroform and then with 50 ml of petroleum ether. A crude petroleum ether extract (50 to 100 mg dry weight) in about 5 ml of petroleum ether was loaded to the column. A stepwise elution was carried out with 50 ml each of petroleum ether, petroleum ether-ethyl ether (90:10, v/v), petroleum ether-ethyl ether (80:20, v/v), and two portions of 50 ml of petroleum ether-ethyl ether (70:30, v/v), while eluate was collected in separate fractions after each change of the solvent.

Thin-layer chromatography

Among many solvent systems tried for both analytical and preparatory silica gel thin-layer chromatography, petroleum ether-ethyl ether-acetic acid (90:10:1, v/v) and petroleum ether-ethyl ether (70:30, v/v) were found to be best suited for the separation of lipid materials in ginseng extract. Although some materials were faintly recognizable as UV-quenching spots at 254 nm, the chromatograms were routinely visualized as fluorescent spots at 375 nm immediately after spraying 0.01 % aqueous solution of rhodamine 6G as described by Marinette (1964). The spots could also be recognized by their pink color even after the rhodamine solution had dried. For preparatory purposes, the material in ethanol or petroleum ether was streaked on either analytical or preparatory plates; the chromatogram was developed by employing either one of the two solvent systems described above; and was sprayed with rhodamine 6G solution. The area of silica gel corresponding to each band was scraped off, and the lipid materials were eluted with ethyl ether three times. Only a trace amount of rhodamine 6G was found in the eluate.

Results

Dried weight of *Panax ginseng* extract and its activity

Dried weight of light petroleum extract from 1g of *Panax ginseng* root was 8 mg to 10 mg, and its inhibitory activity per mg for the growth of L5178Y cells was about 1,000 units. Therefore, the one unit of this activity was equivalent to about 1μg of the extract per ml of culture medium, and the total activities in 1g of the *Panax ginseng* were 8,000 to

Table 2. The growth rate of some cancer and mouse embryo cells

Kind of cells	Incubation time (hours)					Doubling time (hrs.)
	0	12	24	36	48	
Leukemia cell (L5178Y cell)	1.5×10^4 (cells/ml)	3.2×10^4 (cells/ml)	6.2×10^4 (cells/ml)	13×10^4 (cells/ml)	24×10^4 (cells/ml)	12
Sarcoma 180 cell	3.5 × //	4.3 × //	6.6 × //	10 × //	13.5 × //	26
HeLa cell	3.9 × //	5.1 × //	6.2 × //	7.8 × //	9.2 × //	35
Mouse embryo cell	17 × //	/	34 × //	/	69 × //	24

10,000 units.

Estimation of doubling times of some cancer cells and mouse embryo cell

The doubling time of each cell was estimated during 48 hours incubation as illustrated in table 2. The doubling times of three cancer cells, the leukemia cells, Sarcoma 180 cells and HeLa cells were 12, 26 and 35 hours, respectively, and that of the mouse embryo cells (primary cultured) was 24 hours.

Inhibition of L5178Y cell growth

Fig. 2 shows that the light petroleum extract of ginseng inhibits the growth of L1578Y cells *in vitro*. When the L5178Y cell was cultured in media containing various amounts (0, 0.5, 1.0, 1.5, and 2.0 units) of the extract during 72 hours, the doubling times of the L5178Y cells were gradually increased according to the concentration of the extract in culture medium. That is, the growth rate of the L5178Y cells was gradually decreased by increasing the concentration of the extract in the culture medium.

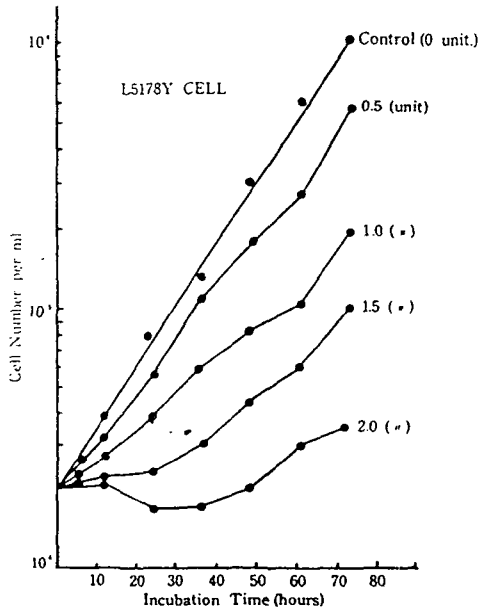

Fig. 2. Growth curves of L5178Y cell in the culture media containing various amounts of the extract of *Panax ginseng*.

Inhibition of HeLa cell growth

Fig. 3. shows that the light petroleum extract of *Panax ginseng* inhibits the growth of HeLa cells *in vitro*. Like the L5178Y cells, the doubling times of the HeLa cells were also gradually increased by increasing of the concentration of the extract in the culture medium, which suggested that the growth of HeLa cells was inhibited by the extract of *Panax ginseng* in the culture medium.

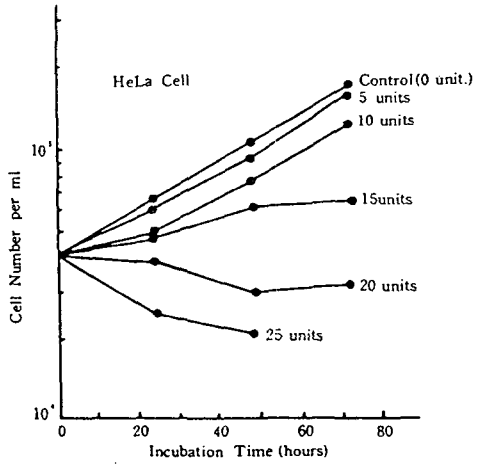

Fig. 3. Growth curves of HeLa cell in the culture media containing various amounts of the extract of *Panax ginseng*.

Comparison of growth inhibitory activities against different cell types

Three different kinds of cells, L5178Y, HeLa

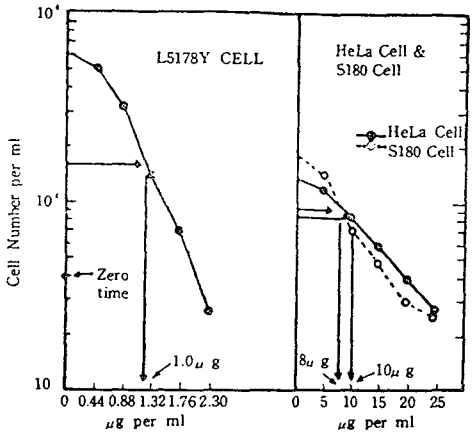

Fig. 4. Comparison of the response of L5178Y(a), Sarcoma 180 and HeLa cells(b) to the extract of *Panax ginseng* after 48 hours of incubation.

and Sarcoma 180 cells were grown in media containing various amounts of the extract. The results shown in Fig. 4a and b indicate that about 8 and 10 folds higher concentrations of *Panax ginseng* extract were required for the Sarcoma 180 cells and HeLa cells, respectively, than for the leukemic cells (L5178Y) to inhibit the cellular growth to the same degree. That is, the doubling time of the L5178Y cells was increased to two folds by incubation for 48 hours in a culture medium containing 10μg of extract per ml, whereas those of Sarcoma 180 cells and the HeLa cells were increased to the same degree by 8 μg/ml and 10μg/ml, respectively, as indicated in Fig. 4a and b.

The response of mouse embryo cells to *Panax ginseng* extract

The *Panax ginseng* extract did not indicate any effect on the growth of the mouse embryo cells in media containing various amounts (1.45 μg to 30 μg per ml) of the extract as shown in Table 3. These amounts (maximum 30 μg) of the extract were equivalent to about 30-fold higher concentration compar with the amounts of the extract for two folds increase of the cellular doubling time of L5178Y cells.

Partial purification of cytotoxic compound

A crude petroleum ether extract from 10 g of ginseng roots (100 mg, 1,000 units/mg) was subjected to a silicic acid column chromatography as described in the method section above. The results are shown in Table. 4.

Table 3. The effect of *Panax ginseng* extract against growth of the mouse embryo cell

Extract added	Incubation time (hours)		Extract added	Incubation time (hours)	
(μg/ml)	0	24	(μg/ml)	0	24
0 (control)	5.5×10^5 (cells/ml)	9.11×10^5 (cells/ml)	0 (control)	5.90×10^4 (cells/ml)	1.45×10^5 (cells/ml)
1.45	//	9.56 //	6	//	1.54 //
2.91	//	8.75 //	12	//	1.37 //
4.36	//	8.96 //	18	//	1.54 //
5.82	//	9.33 //	24	//	1.13 //
7.27	//	8.14 //	30	//	1.48 //

Table 4. The cytotoxic activity of each fraction separated by silicic acid column chromatography

Fraction	Activity per mg	Total activity	Recovery
Crude extract	1,000 units/mg	96,000 units	/
Fraction No. 1	No activity	/	/
No. 2	No activity	/	/
No. 3	900 units/mg	9,600 units	10%
No. 4	3,900 units/mg	44,160 units	46%
No. 5	50 units/mg	1,920 units	2%

Crude Extract: Petroleum ether extract from *Panax ginseng* powder
Fraction No. 1: Only petroleum ether fraction
No. 2: Pet. ether: ethyl ether; 90:10 (v/v) fraction
No. 3: // : // 80:20 //
No. 4: // : // 70:30 //
No. 5: Only ethyl ether fraction

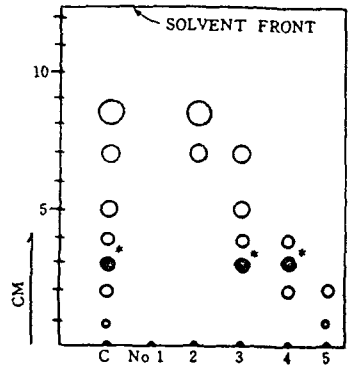

Fig. 5. Silica-gel thin-layer chromatography of crude extract and purified ginseng extract.
C: crude extract of ginseng extract.
No 1: petroleum ether fraction of silicic acid column.
2: pet. ether: ethyl ether (90:10) //
3: // : // (80:20) //
4: // : // (70:30) //
5: ethyl ether fraction
*: active compound

Typical thin-layer chromatorgrams of the crude extract and fractions from the silicic acid column chromatography are shown in Fig.5.

The Rf values of active compounds were about 0.3 with petrolenm ether-ethyl ether-acetic acid (90:10:1, v/v) solvent system.

The cytotoxic activity of the extract against Sarcoma 180 cells *in vivo*

The partial purified extract (7:3 fraction from silicic acid column) was dried by evaporator under nitrogen gas and dissolved in small amount of ethyl alcohol, and then the alcoholic solution was filtered by millipore filter disc under sterile conditions after dilution with distilled water (alcohol cont., less than 0.2%).

Swiss mice used as experimental animals were divided into two groups: control and experimental groups (each 10 heads), and they were inoculated with 1.96×10^5 cells of Sarcoma 180.

The sterilized extract (3,200 units/head/day) was injected to abdomen of the experimental group for 10 days from 3rd day to 12th day after cell inoculation.

The survival time of the experimental group was extended more 1.5 to 2 times than the control group as shown in Fig. 6.

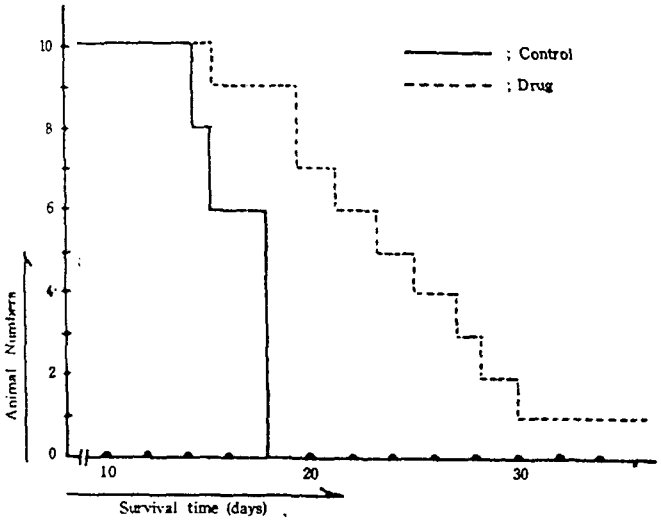

Fig. 6. Survival time of the control and experimental groups.

Discussion

It has been known that *Panax ginseng* has various pharmacological activities through many reports during the past decade. The lipid soluble compounds in dried ginseng root that inhibit the growth of cancer cells of three kinds, mouse leukemic lympoblast cells(L5178Y), Sarcoma 180 cells and HeLa cells *in vitro*, and the growth of Sarcoma 180 cells *in vivo* were clearly demonstrated in this study.

It was demonstrated that the extract did not show any effect on the growth of mouse embryo cells (as normal cells) in a medium containing a high concentration(30μg/ml) of the extract.

Brekhman (1966) and Han (1974) reported that the effective compounds in *Panax ginseng* for pharmacological activity would be a ginsenoside (panax saponin), which is soluble in water, but the compound from *Panax ginseng* used in this experiment was a lipid soluble material extracted by light petroleum, because our primary data show that water extract of *Panax ginseng* does not have any activity to inhibit mouse leukemic cellular growth (L5178Y) *in vitro*.

Hence, although the water soluble extracts (Panax saponin) from *Panax ginseng* were valuable for some pharmacological activity, it must be con-

sidered that the light petroleum extracts show inhibitory activity on the growth of three kinds of cancer cells *in vitro* and Sarcoma 180 cells *in vivo*. And it is also appreciated that 8 to 10 mg of light petroleum extract as dried weight can be obtained from 1g of *Panax ginseng*, because the extract contained the activity of 1,000 units per mg.

However, it is due to some possible selective toxicity that its effectiveness as growth inhibitor against three different types of cells varied by a factor of 8 and 10 folds. The data presented in Fig. 4a and b indicate that about 8 and 10 folds higher concentration of ginseng extract were required for the Sarcoma 180 cells and HeLa cells, respectively, than for the leukemic cells (L5178Y) to inhibit cellular growth to the same degree.

Although the main reason for the variation of the response of the different cells to extract is not clear, it may be due to the length of doubling time of the each kind cell because the doubling time of L5178Y is 12 hours, while that of Sarcoma 180 and HeLa cells are 26 and 35 hours, respectively.

It is very interesting results that cytotoxic activity of the active compound show the positive data as activity of the active compound as growth inhibitor on the Sarcoma 180 cell *in vivo* (Fig. 6) and the negative data on the normal cells (primary culture of mouse embryo cells, Table 3).

For this compound, the aspects of immediate interest include study to determine the mechanism of its cytotoxic action.

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