Korean J. Ginseng Sci.
Vol. 22, No. 1, 18~21(1998)

Comparative Cytotoxic Activities of Various Ginsengs on Human Cancer Cell Lines

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(Received December 5, 1997)

Abstract : Comparative cytotoxic activities of petroleum ether soluble fraction from various ginsengs of Panax species were evaluated using A549 (human lung adenocarcinoma) and SK-OV-3 (human ovary carcinoma) cancer cell lines. Korean red ginseng, Korean white ginseng, American ginseng and Canadian ginseng were found to show more potent cytotoxicities on A549 and SK-OV-3 cell lines than Chinese red ginseng, Japanese red ginseng and Sanchi ginseng. It is noteworthy that especially, red ginseng prepared from the root of *Panax ginseng* cultivated in Korea shows relatively stronger cytotoxic activities than those cultivated in China and Japan.

Key words : *Panax ginseng*, various ginsengs, petroleum ether soluble fraction, cytotoxicity, human cancer cell lines.

Introduction

Ginseng has been traditionally used as a precious drug in oriental countries such as Korea, China and Japan for more than 5,000 years. The source plant of ginseng is *Panax ginseng* C.A.Meyer (Araliaceae), a herb with fleshy roots which grows wild in cool and shady forests extending from Korea and north eastern China to far eastern Siberia. However, because wild ginseng is relatively rare and very expensive, it has been cultivated in Korea, China and Japan. Most of commercially available ginseng is the root of *P. ginseng* cultivated in Korea, the northeast district of China and Japan. The root of *Panax ginseng* is steamed and dried to prepare red ginseng, while the peeled roots dried without steaming are designated as white ginseng. The commercially available ginseng roots are classified into two forms, red and white ginsengs. On the other hand, three closely related plants, American and Canadian ginsengs (*Panax quinquefolium*) and Sanchi ginseng (roots of Panax notoginseng, Yunnan, China) are also used for similar medicinal purpose. American and Canadian ginsengs are root of *Panax quiquefolium* L., growing wild in the northeastern part of the United States and the eastern part of Canada, respectively and now being cultivated. Sanchi ginseng is root of *Panax notoginseng* (Burk.) F.H. Chen, cultivated in the southwestern part of China, Yunnan, Kwansi and some part of Vietnam. Korean ginseng has been accordingly faced on the outside challange to its reputation in that ginsengs cultivated in China and Japan are the same species as Korean ginseng and may have similar biological activities. Since the anticancer activity of petroleum ether soluble fraction from the root of *P. ginseng* has recently been reported.[1-5] Several scientists have recently isolated polyacetylene compounds responsible for the growth inhibition of cancer cell lines.[6-8] Hwang *et al.*[9] have recently reported that growth inhibitory effects of Korean red ginseng extracts were significantly greater than that of Chinese red ginseng. However, the comparative cytotoxic activities of various gin-

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sengs have been not yet extensively reported. This finding has led to the further study of the comparative anticancer effects of petroleum ether soluble fraction from various ginsengs. Therefore, we report herein the comparative cytotoxic activities of various ginsengs on A549 (human lung carcinoma) and SK-OV-3 (human ovary adenocarainoma) cell lines.

Materials and Methods

1. Plant material

The plant material are as follows. Korean red ginseng; Panax ginseng cultivated for six years, Suwon experimental station, Korea and processed according to the GMP of Korea Tobacco & Ginseng Corporation, Korean white ginseng; *Panax ginseng* cultivated under the same conditions as descrived above and dried without peeling, Chinese red ginseng; *Panax ginseng* cultivated for six years in Jilin, China and processed, Japanese red ginseng; *Panax ginseng* cultivated for six years in Shinshu, Japan and processed, American ginseng; *Panax quinquefolium* cultivated for four years in northerneast area, U.S.A. and dried, Canadian ginseng; *Panax quinquefolium* cultivated for four years in Ontario, Canada and dried, Sanchi ginseng; *Panax notoginseng* cultivated for four years in Yunnan, China and processed.

2. Preparation of petroleum ether soluble fractions from various ginsengs

Each fifty grams of the root from various ginsengs was extracted with 80% methanol refluxing at 75℃ two times. After concentration under reduced pressure, the MeOH extract was suspended in water and then partitioned into petroleum ether. Removal of the solvent under reduced pressure provided an petroleum ether soluble fraction, which was used for biological assay.

3. Cytotoxic assay

A549 (human lung carcinoma) and SK-OV-3 (human ovary adenocarcinoma) were grown in RPMI 1640 medium with 5% fetal bovine serum. The initial concentration of each cells in medium was adjusted to 5×10^4 cells/m*l*, and incubated for 48 h. at 37 ℃ under the 5% CO_2. For cytotoxicity assay against A549 and SK-OV-3 cell lines, 1×10^5 cells in 1 m*l* of the medium were seeded into each well of 24 well plates, and preincubated for 24 h. at 37℃ under 5% CO_2, followed by incubation with varying concentration of the extracts for 48 h. The experiment was carried out according to the sulforhodamin B method of the NCI protocol.[10] Cytotoxicity of the extracts at various concentrations against each cell line was calculated as the net growth inhibition (%) of cells as compared with that of control.

Results and discussion

As a part of the studies on the comparative biological activities of various ginsengs, the cytotoxic assay on petroleum ether soluble fractions, showing anticancer effects of ginseng, of Panax species was basically was carried out making use of A549 and SK-OV-3 cancer cell lines. The roots of *Panax* species were extracted with 80% MeOH and the extracts were partitioned with petroleum ether to give petroleum ether soluble fractions for cytotoxic assay. At the concentration of 80 μg/m*l* of petroleum ether soluble fraction, marked differences of inhibitory effects on the growth of cancer cell lines (A549, human lung carcinoma) were observed among various ginsengs tested.

As shown in Table 1, Korean red ginseng (KRG), Korean white ginseng (KWG), American ginseng (AG) and Canadian ginseng (CA) showed above 93% inhibition, while Chinese red ginseng (CRG), Japanese red ginseng (JRG) and Sanchi ginseng (SG) did weaker cytotoxicity (57 to 70% inhibition) than KRG, KWG, AG and CA. This result is partially in good agreement with earlier result of Hwang *et al.*[9] using P388 (mouse leukemia cell line), HT-29 (human colon carcinoma cell) and HRT-18(human rectal carcinoma cell). Interestingly, KRG and KWG were found to show the most potent cytotoxicities (96.5% and 97.2% inhibition, respectively) about 1.5 times as strong as CRG and JRG. And also, at the concentrations

Table. 1. Cytotoxic activities of petroleum ether soluble fractions in Panax genus on A549 (human lung carcinoma) cell line

Concentration (ug/ml)	*P. ginseng*				*p.quinquefolium*		*p.notoginseng*
	KRG[a)]	CRG	JRG	KWG	AG	CG	SG
10	13.22±2.28[b)]	7.34±1.45	11.21±3.25	17.30±4.28	13.45±3.12	19.10±5.80	8.00±1.32
20	34.57±4.35	12.47±2.54	18.02±6.47	37.11±8.56	39.23±6.48	48.41±6.33	14.74±2.41
40	72.43±8.28	30.74±4.83	45.87±14.25	76.69±9.26	73.85±12.42	80.41±7.79	43.21±9.32
80	96.50±10.45	57.12±8.54	66.27±8.31	97.20±13.54	95.87±12.76	93.94±17.25	66.77±8.46

[a)] KRG; Korean red ginseng, CRG; Chinese red ginseng, JRG; Japanese red ginseng, KWG; Korean white ginseng, AG; American ginseng, CG; Canadian ginseng, SG; Sanchi ginseng

[b)] Each value represents the average±standard error of triplicate experiments and is expressed as inhibition ratio(%) of cell grouth.

Table. 1. Cytotoxic activities of petroleum ether soluble fractions in Panax genus on SK-OV-3 (human day carcinoma) cell line

Concentration (ug/ml)	*P. ginseng*				*P. quinquefolium*		*P. notoginseng*
	KRG[a)]	CRG	JRG	KWG	AWG	CWG	SG
10	0	0	0	12.60[b)]±2.54	10.88±1.39	17.23±1.84	0
20	16.62±5.48	5.41±1.58	8.25±1.44	25.73±2.52	24.93±3.41	38.71±4.63	8.58±1.23
40	60.41±8.48	26.56±3.49	30.08±5.48	69.95±8.62	68.47±8.25	71.98±11.25	32.53±8.24
80	96.02±11.23	50.95±8.25	57.82±7.48	96.48±10.44	94.34±10.43	95.61±9.45	67.47±11.44

[a)] KRG; Korean red ginseng, CRG; Chinese red ginseng, JRG; Japanese red ginseng, KWG; Korean white ginseng, AG; American ginseng, CWG; Canadian white ginseng, SG; Sanchi ginseng

[b)] Each value represents the average±standard error of triplicate experiments and is expressed as inhibition ratio(%) of cell growth

ranging from 10 to 40 μg/ml, the petroleum ether soluble fractions of various ginsengs exhibited a similar trend in the order of KRG, KWG, AG and CA as the case for the concentration of 80 μg/ml.

In our parallel study, we have also examined the cytotoxicities of various ginsengs on SK-OV-3 (human ovary carcinoma) cell line. As shown in Table 2, KRG, KWG, AG and CA were also found to exhibit more potent cytotoxicity (above 94% inhibition) at the concentration of 80 μg/ml. However, CRG, JRG and SG were found to show weaker cytotoxicity (50 to 67% inhibition) than the above mentioned ginsengs. Furthermore, at the concentration of ranging from 10 to 40 μg/ml, it was also found that they exhibited mostly the same as those on the growth of A549 cell line. From the above results, it is very difficult to give any definite conclusion on a siginificant difference of cytotoxicities among various ginsengs, however, it is suggested that especially, red ginseng prepared from the root of *Panax ginseng* cultivated in Korea show relatively stronger cytotoxic activities than that cultivated in China and Japan. Anyway, Further experiments have to be performed in order to fully confirm the comparative cytotoxic activities among various ginsengs of *Panax* species.

요 약

고려인삼을 포함한 각국삼의 석유에텔 가용성 분획에 대해 인체 폐암세포인 A549와 인체 자궁암세포인 SK-OV-3를 대상으로 세포독성효과를 비교하였다. 고려홍삼 및 백삼, 미국삼이 중국홍삼, 일본홍삼 및 전칠삼보다 강한 세포독성효과를 보여주었다. 또한, 특징적인 것은 한국에서 재배된 고려인삼으로 제조한 고려홍삼이 중국 및 일본에서 재배한 동일한 홍삼보다 강한 세포독성효과를 보여주었다.

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