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Studies on the Mechanism of Cytotoxicities of Polyacetylenes against L1210 Cell

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Abstract□This study was performed to investigate the mechanism of *in vitro* cytotoxic actions of polyacetylenes which are panaxydol, panaxynol and panaxytriol isolated from *Panax ginseng* C.A. Meyer. DNA synthesis of L1210 cells was significantly inhibited with dose-dependent pattern when L1210 cells were treated for 1 hour with over 5 μg/m*l* of polyacetylenes. Panaxydol which had the most potent cytotoxicity among three polyacetylenes showed also the strongest inhibitory effect on DNA synthesis. Intracellular cyclic AMP levels of L1210 cells treated with 2.5 μg/m*l* of panaxydol or panaxytriol were significantly elevated on the incubation duration. The elevation of cyclic AMP levels by panaxytriol was higher than that by panaxydol, but no significant increase in cyclic AMP by panaxynol was observed. All three polyacetylenes had no effect on glycolysis of L1210 cells. Electron microscopic observations revealed that polyacetylenes caused damage to plasma membranes of L1210 cells in proportion to their cytotoxicities at each ED_{50} value (panaxydol > panaxynol > panaxytriol). These results suggest that cytotoxicities of polyacetylenes against L1210 cells might be mediated by elevated cyclic AMP level, even though the relationship among their cytotoxicities, inhibitory effect on DNA synthesis and ability to elevation of cyclic AMP level are not fully agreed, and might be also related to membrane damage.

Keywords□polyacetylene, panaxydol, panaxynol, panaxytriol, cytotoxicity, *Panax ginseng* C.A. Meyer, L1210 cell, DNA synthesis, cyclic AMP, glycolysis, membrane damage.

It was reported that petroleum ether extract of ginseng inhibited the growth of L5178Y, L1210, Sarcoma 180, HeLa and human cancer cell lines *in vitro*[1-5] and showed antitumor activity *in vivo*[1,3,5]. But active substances responsible for these effects were not well known.

Recently panaxydol was first found to have a very strong cytotoxic activity against L1210 cell by Ahn[6] and followed by isolations of a series of polyacetylenes which showed cytotoxicities against L1210 cell; The C_{17}-polyacetylenes are panaxynol[7], panaxytriol[7], heptadeca-1, 8-dien-4, 6-diyn-3, 10-diol[8], acetyl panaxynol[9], panaxydol chlorohydrin[9] and 10-acetyl panaxytriol[10,11] and the C_{14}-polyacetylenes are panaxyne[11] and panaxyne epoxide[12].

Also Katano[13] reported that panaxytriol from *Panax ginseng* inhibitied the growth of murine lukemia and human cancer cells. Panaxacol and dihydropanaxacol isolated from Ginseng callus showed cytotoxicities against Yoshida Sarcoma 180 cell[14].

Pharmacological and biological activities of polyacetylenes with cytotoxicities against cancer cell lines have been known only little. Panaxynol isolated ginseng root showed anti-inflamatory effect[15]. Falcarindiol, heptadeca-1,8-dien-4,6-diyn-3,10-diol and panaxynol isolated from Saposhnikovae radix which has been traditionally used as an antiphlogistic, an antipyretic and an analgesic, inhibited the formation of HHT and tromboxan B_2 in human platlets[16]. Falcarindiol isolated from different plants has been exhibited antifungal activity[17,18]. In a very recently study, antioxidant effects of panaxydol, panaxynol and panaxytriol on CCl_4-induced lipid peroxidation were reported[19].

However, no work had been reported for the mechanism of cytotoxic action of polyacetylenes. Therefore, effects of polyacetylenes on macromolecular synthesis as DNA, RNA and protein were reported in the previous study as a nesessary prelimi-

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nary step in elucidation of the mode of action of polyacetylenes[20].

In order to explain in more detail on the mechanism of cytotoxicities of polyacetylenes this study was performed.

EXPERIMENTAL METHODS

Cell culture

L1210 cells (purchased from American Type Culture Collection) were grown as suspension culture in Fisher's medium (GIBCO) in the humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The medium which adjusted to pH 7.2 was supplemented with 10% horse serum (GIBCO), $NaHCO_3$ 1.125 g/*l*, penicillin 100,000 units/*l* and streptomycin 100 mg/*l*. The cells were maintained by transferring twice a week *in vitro*, and in BDF_1 mice by weekly i.p. injections. Cell count was measured by using a hemocytometer.

Isolation of polyacetylenes

The powdered red ginseng was extracted with petroleum ether and concentrated extract was chromatographed over a silica gel column with petroleum ether/ethyl ether as a gradient solvent system. Panaxydol, panaxynol and panaxytriol were isolated by semipreparative HPLC under the following conditions; Column, Allteck NH_2 (250 × 10 mm); Solvent system, Hexane/Isopropyl alcohol (2:1); flow rate, 3.0 m*l*/min; detection, UV 254 nm, and identified with the spectrum of UV, IR, NMR and MS. The chemical structures of polyacetylenes are shown in Table I.

DNA synthesis

3 m*l* of L1210 cell suspension (3.5 × 10^5 cells/m*l*) was incubated with (6-^{3}H)-thymidine (0.1 μCi/m*l*) and various concentration of polyacetylens for 1 hour. The rate of DNA synthesis was measured by the incorperation of radioactive precursor into DNA as described previously[20].

Table I. Structures of polyacetylene compounds

Compound	Structure
Panaxydol	$CH_2=CH\text{-}CH(OH)\text{-}(C\equiv C)_2\text{-}CH_2\text{-}CH\text{-}CH\text{-}(CH_2)_6\text{-}CH_3$ (epoxide O bridging the two CH)
Panaxynol	$CH_2=CH\text{-}CH(OH)\text{-}(C\equiv C)_2\text{-}CH_2\text{-}CH=CH\text{-}(CH_2)_6\text{-}CH_3$
Panaxytriol	$CH_2=CH\text{-}CH(OH)\text{-}(C\equiv C)_2\text{-}CH_2\text{-}CH(OH)\text{-}CH(OH)\text{-}(CH_2)_6\text{-}CH_3$

Determination of cyclic AMP

3 m*l* of L1210 cell suspension (3.5 × 10^5 cells/m*l*) was incubated with or without polyacetylenes (2.5 μg/m*l*) for 30 min., 1, 2, 4 hours. Incubation was terminated by cooling in an ice bath and medium was removed by centrifugation at 600 g for 1 min, then 1 m*l* of 0.1 M HCl was added to the cells which washed twice with 1 m*l* of ice cold saline. HCl extracts of the cells were neutralized with an equal aliquot of 2 times 0.1 M sodium acetate (pH 4.0). Cyclic AMP was determined by means of an assay Kit (Amersharm) based on protein binding method. The radioactivity was measured in toluene/Triton X-100 scintillation solution by liquid scintillation spectrophotometer (Beckman LS1800).

Determination of lactate

2 m*l* of L1210 cell suspension (1 × 10^6 cells/m*l*) was incubated with or without polyacetylenes for 1, 2, 4 and 6 hours. Incubations were stopped by adding 4 m*l* of 8% (w/v) perchloric acid. The culture tubes were placed in an ice bath for 5 min, and centrifuged. Concentration of lactate in the supernatant was determined enzymatically by using commercial kits (Sigma Chemical Co.).

Electron microscopic observation

5 m*l* of L1210 cell suspension (5 × 10^4 cells/m*l*) was incubated with each dose for 50% growth inhibition (ED_{50} value; panaxydol, 0.03 μg/m*l*; panaxynol, 0.38 μg/m*l*; panaxytriol, 0.42 μg/m*l*) for 48 hours. Also 3 m*l* of cell suspension (1 × 10^6 cells/m*l*) was treated with 10 μg/m*l* of each polyacetylene for 1 hour. After incubation, the cells were washed with ice-cold physiological saline followed by prefixation in 3% glutaraldehyde/1% $OsSO_4$ (1:1, v/v) in 0.1 M sodium cacodylate buffer (pH 7.4) for 30 min. at 4 °C. Postfixation was performed in 0.25% uranyl acetate in 0.1 M acetate buffer (pH 6.3) for 20 min. at 4 °C. The cells washed 3 times with ice-cold physiological saline were embedded in 2% agar, and dehydrated in a series of acetone. Ultrathin sections were made with a ultra microtome (LKB 2088 utratome V), stained with uranyl acetate and lead citrate, and observed with electron microscope (JEOL JEM-100S EM).

RESULTS AND DISCUSSION

Effects of polyacetylenes on DNA synthesis

As shown in Table II, over 5 μg/m*l* of polyace-

Table II. Inhibition of polyacetylenes on DNA synthesis[a] of L1210 cells[b]

Concentration (μg/ml)	Inhibition of isotope intake (%)[c]		
	Panaxydol	Panaxynol	Panaxytriol
2.5	0 ±0.08	0 ±0.05	0 ±0.16
5.0	27.0±4.6*	12.0±4.6*	12.5±4.9*
10.0	44.0±4.2**	34.8±3.7**	35.8±6.8**

[a] DNA-synthesis was determinated by measuring incorperation of (6-^{3}H)-thymidine into ice cold 5% TCA-insoluble precipitations.
[b] L1210-cells in Fisher's medium (3.5×10^{5}ml) were incubated with (6-^{3}H)-thymidine (0.1 μCi/ml) and various concentrations of polyacetylenes for 1 hour.
[c] Inhibition of isotope intake(%) was calculated by using the following equation.
Inhibition of isotope intake(%) =

$$\left(1-\frac{\text{Polyacetylene treated DPM}}{\text{Control DPM}}\right)\times 100$$

* $p<0.05$
** $p<0.005$

tylenes inhibited significantly DNA synthesis in a dose-dependent pattern. The inhibitory effects on DNA synthesis were proportional to their potency of cytotoxicity against L1210 cells. Panaxydol having the most potent cytotoxicity among these polyacetylenes showed the strongest inhibitory effects on DNA synthesis. Similar results were found in the previous study[20].

It suggested that the inhibitory effects on macromolecular synthesis such as DNA, RNA and protein were probably associated with the mode of cytotoxic action of polyacetylenes.

Effects of polyacetylenes on intracellular cyclic AMP level

The results given in Table III showed the effects of polyacetylenes on cyclic AMP level involving in the control of cell growth. Cyclic AMP levels of L1210 cells increased almost with increasing incubation time after treatment with polyacetylenes, while cyclic AMP levels of untreated control decreased. The increase in cyclic AMP produced by panaxytriol (ED_{50}, 0.42μg/ml) was higher than the one produced by panaxydol (ED_{50}, 0.03 μg/ml). No significant increase in cyclic AMP produced by panaxynol (ED_{50}, 0.38 μg/ml) was shown. Therefore, it was observed that the ability of polyacetylenes to elevate cyclic AMP might be not corresponded to potency of their cytotoxicities. But our results indicated that these effects of polyacetylnes might, at least in part, play a role in the regulation of cell growth.

A low level of cyclic AMP was found in several types of malignant cells and it was suggested that this low level is responsible for the loss of contact inhibition and for the uncontrolled growth of tumor cells[21-23]. Additions of cyclic AMP, or agents which elevate the intracellular level of cyclic AMP to transformed cells cause growth inhibition and cell kill both *in vitro*[24-26] and *in vivo*[27,28] and in some cases, cause morphological reversion[29,30] and differentiation[31,32]. Further studies on a number of cell line indicated that the intracellular level of cAMP was inversely proportional to the rate of DNA synthesis[33-36].

On the basis of the above findings, considering the relationship between the effects of polyacetylenes on cyclic AMP level and on DNA synthesis in

Table III. Effects of polyacetylenes on the intracellular cyclic AMP levels of L1210 cells[a]

Polyacetylenes	cAMP (pmole/10^{5} cells)[b]			
	30 min	1 hr	2 hr	4 hr
Control	2.44±0.13(100)	2.07±0.13(100)	2.00±0.12(100)	1.74±0.31(100)
Panaxydol	3.07±0.38(126)*	2.51±0.21(121)*	3.11±0.16(156)**	3.38±0.23(194)**
Panaxynol	2.48±0.39(102)	2.11±0.20(102)	2.25±0.36(113)	2.04±0.33(117)
Panaxytriol	3.23±0.18(132)**	2.79±0.25(135)*	3.70±0.10(185)**	3.65±0.15(210)**

a) L1210 cells in Fisher's medium (3.5×10^{5}/ml) were incubated with 2.5μg/ml of polyacetylenes for indicated times.
b) For cyclic AMP measurment cells were fixed by addition 1 ml of 0.1 M HCl immediately after washing 2 times with 1 ml of ice-cold saline. HCl extracts of cells were nutralized with an equal aliquot of 2 times 0.1 M sod. acetate buffer (pH 4.0). cAMP was determined by using cAMP assy kit (Amersharm).
Each value represents mean ± S.E. of 4 determinations. Values in pharenthesis are expressed % of control level.
* $p<0.01$
** $p<0.005$

Table IV. Effects of polyacetylenes on glycolysis of L1210 cells[a)]

Polyacetylenes ($\mu g/ml$)		Lactate production ($\mu g/ml$)[b)] after incubation			
		1 hr	2 hr	4 hr	6 hr
Control		9.77±0.97	26.04±5.21	40.56±6.51	53.38±8.46
Panaxydol	5	10.00±0.98	22.79±2.60	39.06±4.56	49.98±6.51
	10	9.57±1.09	25.39±1.30	39.06±1.95	55.99±3.91
Panaxynol	5	9.77±1.18	22.79±0.85	39.06±1.30	50.13±4.58
	10	10.56±0.97	26.04±3.91	36.33±6.86	51.25±5.20
Panaxytriol	5	9.44±0.38	27.99±2.60	38.41±3.91	52.08±3.26
	10	9.77±0.29	24.09±1.30	38.41±1.95	54.03±5.86

a) 2 m*l* of cell suspension which innoculated 1×10^6 cell/m*l* in fresh Fisher's medium were incubated with polyacetylenes for various time.
b) Lactate was determined enzymatically in the culture medium containing 8% (w/v) perchloric acid.
Each value is mean ±S.E. of 5 results.

the previous study[20)], cyclic AMP levels were elevated after treatment with panaxydol on the incubation duration while DNA synthesis was inhibited at the same concentration of panaxydol. In the cases of panaxynol or panaxytriol a correlation did not appear to exist between elevation of cyclic AMP levels and inhibition of DNA synthesis.

However, the results showing that elevation of cyclic AMP level suggested that cytototxicities of polyacetylenes might be mediated by its effects, even though the relationships between cytotoxicity, elevation of cyclic AMP level and inhibition of DNA synthesis were not fully agreed. In order to investigate the mechanism of elevation of cyclic AMP levels by polyacetylenes further studies is required to elucidate on cyclic AMP system including adenylate cyclase, phosphodiesterase and cyclic AMP-dependent phosphorylation.

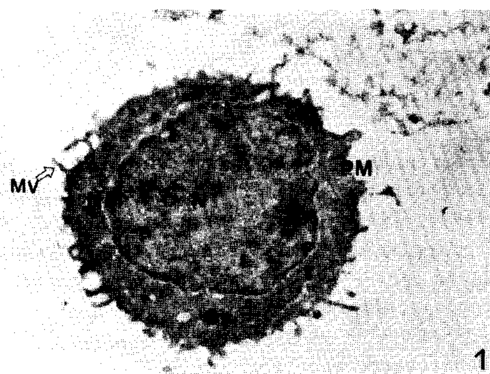

Fig. 1. Electron micrograph of control L1210 cell. (×10,000)

Effects of polyacetylenes on glycolysis of L1210 cells

As shown in Table IV, the production of lactate was increased with growing the cells. These results showed that polyacetylenes had no effect on the glycolysis of L1210 cells, even at a dose of 5 or 10 $\mu g/ml$ which produced potent cytotoxicity.

It was originally proposed by Warburg that the high aerobic glycolysis of tumor cells is caused by a defect in the respiratory activity of the cells[39)]. Racker[40)] proposed that the lesion responsible for rapid lactate formation in tumor cells is a high ATPase activity which supplied ADP and pi required to glycolysis. Also they presented evidance that quercetin a potent inhibitor of cell growth *in vitro* and ouabain a specific ihhibitor of Na^+-K^+ ATPase inhibited aerobic glycolysis in Ehrich ascite tumor cells by reduction of excessive Na^+-K^+ ATPase activity sustain the high aerobic glycolysis[41,42)]. Therefore, our results showed that panaxydol, panaxynol and panaxytriol inhibited the growth of L1210 cells without inhibition of characteristic glycolysis in tumor cells.

Effects of polyacetylenes on morphology of L1210 cell

As shown in Fig. 1, the typical L1210 cell untreated with polyacetylenes possesed a high ratio of nucleus to cytoplasm (N/C ratio) and a thin rim of cytoplasm containing little or no organized endoplasmic reticulum, few mitochondria and a large number of free ribosomes. L1210 cell treated with

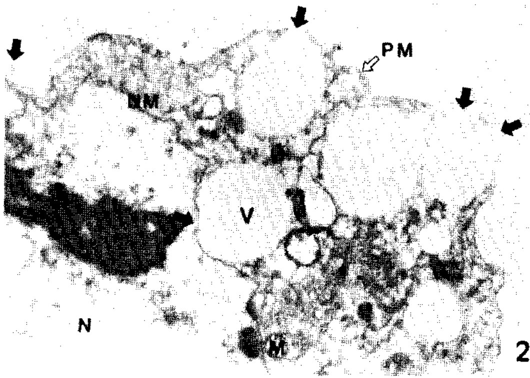

Fig. 2. Electron micrograph of L1210 cell incubated with panaxydol (0.03 μg/ml) for 48 hours. (×12,000)

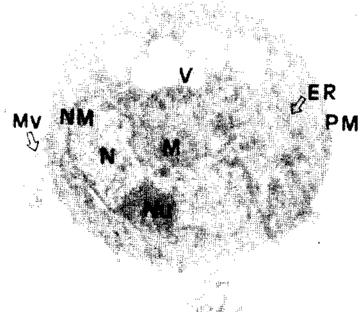

Fig. 4. Electron micrograph of L1210 cell incubated with panaxydol (0.03 μg/ml) for 48 hours. (×10,000)

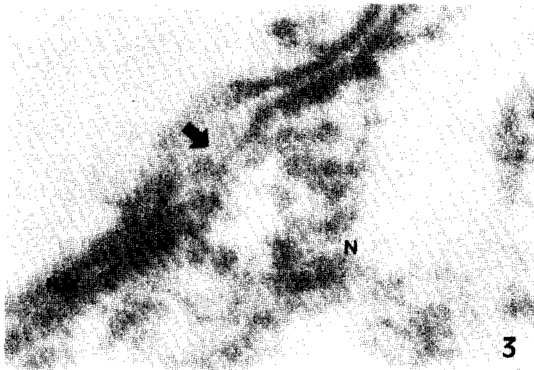

Fig. 3. The partially injured lipid bilaryer of nuclear membrane of L1210 cell incubated with panaxy dol (0.03 μg/ml) for 48 hours. (×200,000)

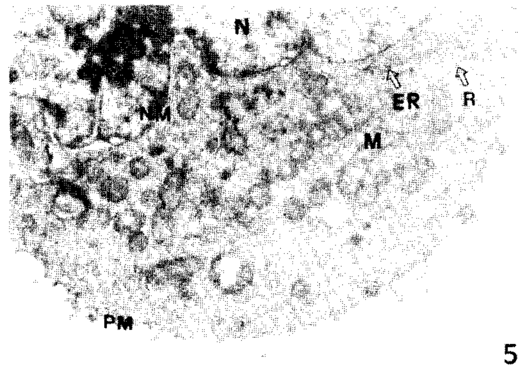

Fig. 5. Electron micrograph of L1210 cell incubated with panaxytriol (0.42 μg/ml) for 48 hours. (×12,000)

0.03 μg/ml of panaxydol for 48 hours in Fig. 2 showed the marked vacuolization, swollen mitochondria and exudation of cytoplasm and nucleoplasm from partially damaged both plasma membrane and nuclear membrane. The partially broken lipid bilayer of nuclear membrane by panaxydol was shown in Fig. 3. When L1210 cells incubated with each ED_{50} value of each polyacetylene respectively, the damages to membranes and mitochondria were observed with increasing their cytotoxicities in order of panaxydol, panaxynol and panaxytriol. The damage to the plasma membrane by panaxytriol was very little. Also morphology of L1210 cell which was not damaged to the plasma membrane by panaxydol or panaxynol was similar to cellular change by panaxytriol. In these cells the N/C ratio decreased markedly and a few vacuole, endoplasmic reticulum, mitochondria and a large

Fig. 6. Electron micrograph of L1210 cell incubated with panaxytriol (10 μg/ml) for 1 hour. (×8,000)

number of free ribosome were existed, as shown in Fig. 4 and 5. On the other hand, when L1210 cells

incubated with 10 μg/ml of each polyacetylene for 1 hour, the degree of damage to the cells was similar each other. As shown in Fig. 6, L1210 cell treated with 10 μg/ml of panaxytriol seems to maintain a cellular shape and plasma membrane and nuclear membrane were partially injured and a few vacuoles and necrosis of cell organelles were found.

It was suggested that antifungal activity of falcarindiol (heptadeca-1,9-dien-4,6-diyn-3,8-diol) was due to its hydrophobic nature forming mixed falcarindiol-phospholipid micelle[18]. Because panaxydol, panaxynol and panaxytriol have a common terminal aliphatic moiety which has a hydrophobic nature, the damage to the membranes showing in our result might be caused by its hydrophobic moiety. It was reported that the potent activity of panaxydol, which is almost 10 times cytotoxic than panaxynol or panaxytriol against L1210 cells, might come from its epoxide[7].

In this study, the correlation between the potency of cytotoxicity and the degree of damage to plasma membrane suggested that structure-activity relationship would exist among polyacetylenes and their cytotoxicities against L1210 cells might also be related to membrane damage.

LITERATURE CITED

1. Hwang, W.I. and Cha, S.M.: A cytotoxic activity of extract of *Panax ginseng* root against cancer cells *in vitro* and *in vivo*. *Proc. 2nd Int. Ginseng Symp.* **43** (1978).
2. Yeon, Y.S., Lee, S.Y., Kim, B.S. and Yun, T.K.: The studies on the mechanism of action of the cytotoxic fraction from Korean ginseng roots. *Korean Biochem. J.* **13,** 203 (1980).
3. Hwang, W.I. and Oh, S.K.: A study on the anticancer activities of lipid soluble ginseng extract and ginseng saponin derivatives against some cancer cells. *Korean J. Ginseng Sci.* **8,** 153 (1984).
4. Lee, S.H. and Hwang, W.I.: Inhibitory effect of petroleum ether extract of *Panax ginseng* root against growth of human cancer cells. *Korean J. Ginseng Sci.* **8,** 141 (1986).
5. Hwang, W.I., Park, K.H. and Paik, J.M.: A cytotoxic activity of *Panax ginseng* extract against some cancer cells *in vivo* and *in vitro*. *Korean J. Ginseng Sci.* **11,** 173 (1987).
6. Ahn, B.Z. and Kim, S.I.: Antineoplastic natural products and the analogues VI. *Arch. Pharm. Res.* **8,** 283 (1985).
7. Ahn, B.Z. and Kim, S.I.: Benziehung zwischen struktur and cytotoxischer aktivitat von panaxydol analogen gegen L1210 zellen. *Arch. Pharm. (Weinheim)* **321,** 61 (1988).
8. Ahn, B.Z. and Kim, S.I.: Heptadeca-1,8-t-dien·4,6-diin-3,10-diol, ein weiteres, gegen L1210-zellen cytotoxisches wirkprinzip aus der Koreanischen Ginseng wurzel. *Planta Medica* **54,** 183 (1988).
9. Ahn, B.Z., Kim, S.I. and Lee, Y.H.: Acetylpanaxydol und panaxydol-chlorohydrin, zwei-neue, gegen L1210-zellen cytotoxische polyine aus Koreanischem Ginseng. *Arch. Pharm. (Weinheim)* **322,** 223 (1989).
10. Kim, S.I., Lee, Y.H. and Kang, K.S.: 10-Acetylpanaxytriol, A new cytotoxic polyacetylene from *Panax ginseng*. *Yakhak Hoeji* **33,** 118 (1989).
11. Kim, S.I., Kang, K.S., Lee, Y.H. and Kim (Jun), H.Y.: *The 37th annual convention of the pharmaceutical society of Korea,* 131 (1988).
12. Kim, S.I., Kang, K.S. and Lee, Y.H.: Panaxyne epoxide, A new cytotoxic polyine from *Panax ginseng* root against L1210 cells. *Arch. Pharm. Res.* **12,** 48 (1989).
13. Katano, M., Yamamoto, H. and Matsunaga, H.: Anti-tumor substance from Korean red ginseng powder. *Proc. 5th Int. Ginseng Symp.* (1988).
14. Fujimoto, Y. and Satoh, M.: Acetylenes from the callus of *Panax ginseng*. *Phytochem.* **26,** 2850 (1987).
15. Otsuka, H., Komiya, T., Fujoka, S., Goto, M., Hiramatsu, Y. and Fujmura, H.: Studies on anti-inflammatory agents. IV. *Yakugaku Zasshi* **101,** 1113 (1981).
16. Baba, K., Tabata, Y., Kozawa, M., Kimura, Y. and Arichi, S.: Studies on chinese traditional medicine "Fan-Feng" (I) Structure and physiological activities of polyacetylene compounds from Saposhnikoviae radix. *Shoyakugaku Zasshi* **41,** 189 (1987).
17. Hansen, L. and Boll, P.M.: Polyacetylenes in araliaceae: Their chemistry, biosynthesis and biological significance. *Phytochem.* **25,** 285 (1986).
18. Garrod, B., Lea, E.J.A. and Lewis, B.G.: Studies on the mechanism of action of the antifungal compound falcarindiol. *New Phytol.* **83,** 463 (1979).
19. Kim(Jun), H.Y., Lee, Y.H. and Kim, S.I.: Effects of polyacetylene compounds from *Panax ginseng* C.A. Meyer on CCl_4-induced lipid pe-

roxidation in mouse liver. *Korea J. of Toxicology* **4,** 13 (1988).
20. Kim, Y.S., Kim, S.I. and Hhan, D.R.: Effect of polyacetylene compound from *Panax ginseng* on macromolecular synthesis of Lymphoid lukemia L1210. *Yakhak Hoeji* **32,** 137 (1988).
21. Otten, J., Johnson, G.S. and Pastan, I.: Cyclic AMP levels in fibroblasts: Relationship to growth rate and contact inhibition of growth. *Biochem. Biophy. Res. Comm.* **44,** 1192 (1971).
22. Sheppard, J.R.: Difference in the cyclic adenosine 3′, 5′-Monophosphate levels in normal and transformed cells. *Nature New Biol.* **236,** 14 (1972).
23. Rein, A., Carchman, R.A., Johnson, G.S. and Paston, I.: Simian virus 40 rapidly lowers cAMP levels in mouse cell. *Bio. Biophy. Res. Comm.* **52,** 899 (1973).
24. Tisdale, M.J. and Phillips, B.J.: Inhibition of cyclic 3′, 5′-nucleotide phosphodiesterase-A possible mechanism of action of bifuntional alkylating agents. *Biochem. Pharmacol.* **24,** 211 (1975).
25. Graziani, Y. and Chayoth, R.: Elevation of cyclic AMP level in Ehrich ascite tumor cells by quercetin. *Biochem. Pharmacol.* **26,** 1259 (1977).
26. Kotoni, M., Koizumi, Y., Yamada, T., Kawasaki, A. and Akabane, T.: Increase of cyclic 3′, 5′-adenosine monophosphate concentration in transplantable lymphoma cells by vinca alkaloids. *Cancer Research* **38,** 3094 (1978).
27. Reddi, P.N. and Constantinides S.M.: Partial suppression of tumor production by dibutyryl cyclic AMP and theophylline. *Nature* **239,** 286 (1972).
28. Webb, D., Braun, W. and Plescia, O.J.: Antitumor effects of polynucleotides and theophylline. *Cancer Research* **32,** 1814 (1972).
29. Johnson, G.S. and Pastan, I.: N^6, $0^{2'}$-dibutyryl adenosine 3′, 5′-monophosphophosphate induces pigment production in melanoma cells. *Nature New Biol.* **237,** 267 (1972).
30. Wijk, R.V., Wicks, W.D. and Clay, K.: Effects of derivatives of cyclic 3′, 5′-adenosine monophosphate on the growth, morphology, and gene expression of hepatoma cells in culture. *Cancer Research* **32,** 1905 (1972).
31. Prasad, K.N., Kumar, S., Gilmer, K. and Vernadakis, A.: Cyclic AMP-induced differentiated neuroblastoma cells: Changes in total nucleic acid and protein contents. *Biochem. Biophy. Res. Comm.* **50,** 973 (1973).
32. Guzit, Y., Ruben, R.C., Deitch, A.D., Marks, P. A. and Rifkind, R.A.: Changes in cyclic Adenosine 3′, 5′-monophosphate levels during induction of differentiation in murine erythroleukemia cells. *Cancer Research* **38,** 3779 (1978).
33. Yang, T.J. and Vas, S.I.: Growth inhibitory effects of adenosin 3′, 5′-monophosphate on mouse lukemia L5178-Y-R cells. *Experimentacia* **15,** 442 (1971).
34. Wijk, R.V., Wicks, W.D., Bevers, M.M. and Rijn, J.V.: Rapid arrest of DNA synthesis by N^6, $0^{2'}$-dibutyryl cyclic adenosine 3′, 5′-monophosphate in cultued hepatoma cells. *Cancer Research* **33,** 1331 (1973).
35. Graziani, Y. and Chayoth, R.: Regulation of cyclic AMP level and synthesis of DNA, RNA and protein by quercetin in Ehrich acites tumor cells. *Biochem. Pharmacol.* **28,** 397 (1979).
36. Tisdale, M.J. and Phillips, B.J.: Comparative effects of alkylating agents and other antitumor agents on the intracellular level of adenosine 3′, 5′-monophosphate in Walker carcinoma. *Biochem. Pharmacol.* **24,** 1271 (1975).
37. Taylor-Papadimitrious, J.: Effects of adenosine-3′, 5′-cyclic monophosphoric acid on the morphology, growth and cell cycle of Earle's L cells. *Int. J. Cancer* **13,** 404 (1974).
38. Burger, M.M., Bombik, B.M., Breckenridge, B.M. and Sheppard, J.R.: Growth control and cyclic alterations of cyclic AMP in the cell cycle. *Nature New Biol.* **239,** 161 (1972).
39. Warburg, O.: On the origin of cancer cells. *Science* **123,** 309 (1956).
40. Racker, E.: Bioenergetics and the problem of tumor gorwth. *Am. Sci.* **62,** 56 (1972).
41. Suolinna, E.M., Lang, D.R. and Racker, E.: Quercetin, and artifical regulator of the high aerobic glycolysis of tumor cells. *J. Natl. Cancer Inst.* **53,** 1515 (1974).
42. Suolinna, E.M., Buchsbaum, R.N. and Racker, E.: The effect of flavonoids on aerobic glycolysis and growth of tumor cells. *Cancer Research* **35,** 1865 (1975).