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EFFECTS OF A CYTOTOXIC SUBSTANCE, PANAXYTRIOL FROM *PANAX GINSENG* C.A. MEYER ON THE IMMUNE RESPONSES IN NORMAL MICE

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ABSTRACT: *The effects of panaxytriol as known to be a cytotoxic substance isolated from Panax ginseng on the immune responses were examined. The i.p. administration of panaxytriol to normal mice for 6 consecutive days as doses of 5, 10 and 20 mg/kg suppressed the increase of body weight dose-dependently but did not affect the weight ratio of immunoorgans to body weight. No significant changes were observed in the humoral immune responses as measured by Arthus reaction and plaque forming cells and in the cellular immune response as measured by delayed hypersensitivity as well as phagocytic activity of reticuloendotherial system. These results suggested that panaxytriol, a cytotoxic substance to cancer cells, has no detrimental effects on the immune function in normal mice.*

Keywords: *Panaxytriol, Panax ginseng, Arthus reaction, Delayed hypersensitivity, Plaque forming cell, Phagocytic activity, Reticuloendotherial system*

INTRODUCTION

In recent years, series of polyacetylenes including the C17-and the C14-compounds isolated from *Panax ginseng* C.A. Meyer have been exhibited *in vitro* cytotoxicities against L1210 cells (Ahn and Kim, 1988a, b, 1989; Kim *et al.*, 1989 a, b, c). Among these polyacetylenes, Panaxytriol was reported to be isolated as one of the characteristic constituents of Ginseng Radix Rubra (Kitakawa *et al.*, 1983, 1987), and to inhibit the growth of various kinds of human cultured cancer cell lines, B16 mouse melanoma, mouse fibroblast-derived tumor L929 (Matsunaga *et al.*, 1989) and the solid form of sarcoma 180 cells *in vivo* (Kim, 1988).

It was reported that the mode of cytotoxic action of polyacetylenes, such as panaxydol, panaxynol or panaxytriol might be mediated by elevation of intracellular cAMP levels in L1210 cells and was related to the inhibition of DNA, RNA and protein synthesis and also damage to membranes (Kim *et al.*, 1988, 1989d). On the basis of

possible mode on cytotoxic action of polyacetylenes, it could be considered that polyacetylenes are directly cytotoxic to cancer cells. Cytocidal anticancer chemotherapeutics are known to have detrimental side effects and destory host defence mechanisms (Kauffman and Foye, 1981).

Therefore, it was examined whether panaxytriol showing cytotoxicities against cancer cells effects on immune functions in normal mice.

MATERIALS AND METHODS

Animals

Male ICR mice, 5-6 weeks old, weighing 20-24g were supplied from Animal Breeding Room of the Korea Ginseng and Tobacco Research Institute. They were housed in a room at 22±2°C with 55±5% relative humidity and given a defined laboratory rodent chow (Sam Yang Inc. Ltd.) and tap water *ad libitum*.

Isolation of Panaxytriol from *Panax ginseng* C.A. Meyer

Panaxytriol (Fig. 1) was isolated from petroleum ether extract of Korean Red Ginseng Roots (obtained from Korea Tobacco and Ginseng Corp.) by the method of Ahn and Kim (1988a).

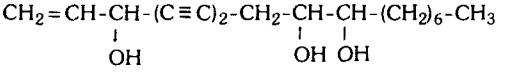

Fig. 1. The chemical structure of panaxytriol

Sample Administration and Immunization

Panaxytriol dissolved in 50% (v/v) PEG 400/physiological saline was administered intraperitoneally for 6 consecutive days to normal male ICR mice at doses of 5, 10 and 20 mg/kg. Sheep red blood cells (SRBC, purchased from Korea Media Co. Seoul, Korea) were used as the antigen, which was stored in Alserver's solution at 4-6°C, were washed four times with Hank's Balanced Salt Solution (HBSS, pH 7.2) by the centrifugation at 400×g for 5 min. and the SRBC suspension was prepared. At the next day after the first administration, mice were sensitized with 0.1 m*l* of SRBC suspension (1×10^8 cells/m*l*) into the tail vein and after one day of the last administration, challenged intradermally with 0.05 m*l* of SRBC suspension (2×10^9 cells/m*l*) into the foot pad.

Arthus Reaction (AH) and Delayed Hypersensitivity (DH)

To determine AH and DH, foot pad swelling thickness was measured with micrometer (Mistutoyo, Japan) at 3 hr. and 24 hr. after challenge, respectively. Foot pad swelling index was calculated using the following equation.

$$\text{Foot pad swelling index} = \frac{\text{Swelling thickness of foot pad (after challenge-before challenge)}}{\text{Thickness of foot pad before challenge}} \times 100$$

Preparation of Spleen Cell Suspension

The spleen was aseptically removed from each mouse sacrificed by cervical dislocation, gently teased between the frosted ends of two microscope slides and passed through a nylon mesh in ice-cold HBSS. After centrifugation at 400g for 5 min., cell pellet was suspended in 5 m*l* of Tris-buffered 0.83% NH_4Cl (pH 7.2) and incubated at 37°C for 5 min. The single cells obtained were washed twice with ice-cold HBSS by centrifugation at 400 × g for 5 min., resuspended and counted using a hemacytometer.

Hemolytic Plaque Forming Cell(PFC) Assay

At the 8th day, one day after challenge, the PFC response to SRBC was determined by the method of Chunningham and Szenberg (1968) with a slight modification. Two microchambers whose capacity was 45 μl each, covered with coverglass, were made on the slide glass. The suspension of indicator red cells in HBSS was prepared with 500 μl of 50% (v/v) SRBC and 1000 μl of guinea pig complement (Sigma Co.) and stood in an ice bath for 30 min. Reaction mixture was composed of 435 μl of spleen cell suspension and 100 μl of suspension of indicator red cells, packed into microchambers and then microchambers were sealed with paraffin/wax(1:1). After incubation at 37°C for 1 hr., the number of hemolytic plaques was determined under a low power microscope. PFC/10^6 spleen cells and PFC/spleen were calculated by the following equations.

$$\text{PFC}/10^6 \text{ spleen cells} = \frac{N}{C.Vm.a} \times 10^6$$

$$\text{PFC/spleen} = \frac{\text{PFC}}{10^6 \text{ spleen cells}} \times C.Vs.$$

a : the ratio of spleen cell suspension in reaction mixture (435/535)
N : the number of plaque observed in a microchamber
C : the count of spleen cells in 1 m*l* of spleen cell suspension
Vm : the volum of incubation mixture filled into a microchamber
Vs : total volume of spleen cell suspension (m*l*)

Phagocytic Activity

Phagocytic function was measured by the method of Biozzi *et al.* (1954). At one day after the last sample administration, a suspension of carbon particle (Rotring Drowing Ink., Germany) in 1% gelatin solution (1:5) was injected intravenously (0.1 m*l*/10g body weight). After 10, 20 and 30 min. of carbon injection, 20 μl of blood was taken from the retro-orbital plexus with heparinized capillary and transferred into 2m*l* of 0.1% Na_2CO_3. The absorbance at 600 nm was measured. Phagocytic index (K) and corrected phagocytic index(a) were calculated by means of the following equations.

$$K = \frac{\log OD_{t1} - \log OD_{t2} \cdot \alpha}{t2 - t1}, \quad \alpha = \sqrt[3]{K} \cdot \frac{Pc}{Po}$$

OD1, OD2: the optical density at time t1 and time t2, respectively
Pc: body weight
Po: liver weight + spleen weight

Statistical Analysis

Statistical significance was evaluated by Student's t-test.

RESULTS AND DISCUSSION

When panaxytriol was administered i.p. to normal ICR mice at doses of 5, 10 and 20 mg/kg for 6 consecutive days, as shwon in Fig. 2, the increase of body weight was

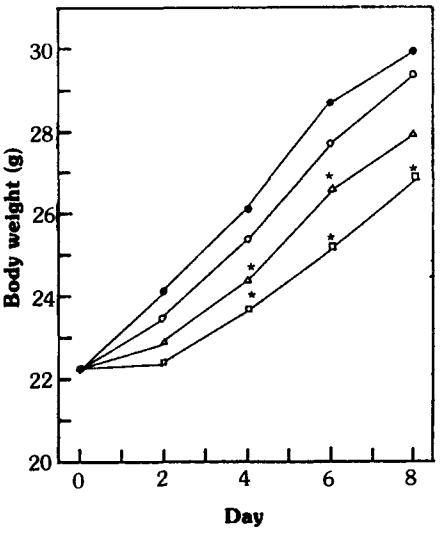

Fig. 2. Effect of panaxytriol on body weight in normal male ICR mice.
Panaxytriol was administered intraperitoneally for 6 consecutive days from day 1 to day 6.
●—● : control
○—○ : panaxytriol 5m/kg/day
△—△ : panaxytriol 10mg/kg/day
□—□ : panaxytriol 20mg/kg/day
Each value represents mean ± S.E. of 6 mice
*$p < 0.05$ vs. control group.

Table 1. Effect of panaxytriol on immunoorgans in normal male ICR mice

Group	Dose (mg/kg/day)	No. of mice	Immunoorgan/Body weight (%)		
			Liver	Spleen	Thymus
Control	—	6	7.02 ± 0.45	0.62 ± 0.08	0.26 ± 0.09
Panaxytriol	5	6	6.82 ± 0.26	0.70 ± 0.11	0.24 ± 0.07
	10	6	6.79 ± 0.26	0.65 ± 0.13	0.27 ± 0.07
	20	6	6.67 ± 0.47	0.69 ± 0.05	0.28 ± 0.05

Panaxytriol was administered intraperitoneally for 6 consecutive days.
After 2 day of the last sample administration immunoorgans were measured.
Each value represents mean ± S.E.

suppressed dose-dependently as compared with the control group.

Table 1 showed that the weight ratio of liver, spleen or thymus to body weight was not altered by treatment of panaxytriol.

As shwon in Table 2, panaxytriol did not produce any significant changes in both AH, which is immune complex mediated hypersensitivity at 3 hr. and DH, which is mediated primarily by T cells and macrophages at 24 hr. after challenge. Also DH is strongly regulated by suppressor T lymphocytes. It was reported that cyclophosphamide depress the humoral immune response severely, while the cell-mediated immune response such as DH is enhanced by selective inactivation of suppressor T cells (Ha and Jung, 1977, 1979). Therefore, these data indicated that panaxytriol might not affect on immunologically-mediated inflammation and suppressor T cells among T cell subpopulations.

As shown in Table 3, there were no significant differences in the number of hemolytic

Table 2. Effect of panaxytriol on the Arthus reaction and delayed hypersensitivity in normal male ICR mice

Group	Dose (mg/kg/day)	No. of mice	Foot Pad Swelling Index	
			AH (3hr)	DH (24hr)
Control	—	10	11.95±4.40	9.97±4.11
Panaxytriol	5	10	10.39±4.43	8.40±4.53
	10	10	12.13±4.11	8.20±4.57
	20	10	11.22±4.27	8.20±4.82

Panaxytriol administered intraperioneally for 6 consecutive days (day 1-6).
On day 2, mice were sensitized with 1×10^7 SRBC into the tail vein and challenged with intradermally 1×10^8 SRBC in the foot pad on day 7, and foot pad swelling was measured at 3 hr and 24 hr after challenge, respectively.

$$\text{Foot pad swelling index} = \frac{\text{Swelling thickness of foot pad (after challenge-before challenge)}}{\text{Thickness of foot pad before challenge}} \times 100$$

Each value represents mean ± S.E.

Table 3. Effect of panaxytriol on hemolytic plaque forming cells in normal male ICR mice

Group	Dose (mg/kg/day)	No. of mice	Total spleen cell ($\times 10^7$)	PFC/10^6 spleen cells	PFC/spleen ($\times 10^2$)
Control	—	6	11.38±2.55	42.3± 9.6	47.9±18.1
Panaxytriol	5	6	12.01±2.40	39.1±14.1	45.4±14.6
	10	6	10.46±1.95	35.8±19.5	34.6±15.2
	20	6	10.89±1.94	42.1±19.9	38.3±12.6

Panaxytriol was administered intraperitoneally for 6 consecutive days (day 1-6).
On day 2 mice were sensitized with 1×10^7 SRBC into the tail vein, on day 7 challenged with 1×10^8 SRBC in the foot pad, and on day 8 PFCs were determined by Cunningham's method with a slight modification. Each value represents mean ± S.E.

PFC among the groups. Futhermore, total number of spleen cells was equal between the control group and the treated group. These results would relate to the report that panaxytriol did not inhibit the growth of normal cells such as human fibroblast, MRC-R cells (Mastunaga *et al.*, 1989). From these results, it was suggested that panaxytriol might not induce cytotoxicity against spleen cells *in vivo* and also has no effects on antibody-secreating cells to SRBC.

The effect of panaxytriol on nonspecific phagocytic activity of reticuloendotherial system was presented in Table 4. The corrected phagocytic index tended to decrease in a dose dependent manner but was not statistically significant. It suggested that panaxytriol might not depress phagocytosis of splenic sinus macrophages and kuffer cells in the livers.

The above results showing no significant alteration in the various immune responses suggested that panaxytriol, which has cytotoxicities against cancer cells like other directly cytocidal anticancer agents, might not cause the immunotoxicity within the dose of 20 mg/kg which showed antitumor activity against the solid form of sarcoma 180.

In conclusion, panaxytriol might not suppress both the humoral and cell-mediated immune function as well as phagocytosis of reticuloendotherial system.

Table 4. Effect of panaxytriol and phagocytic activity in normal male ICR mice

Group	Dose (mg/kg/day)	No. of mice	Phagocytic Index (10^{-2})	Corrected Phagocytic Index
Control	—	4	1.43±0.22	3.75±0.36
Panaxytriol	5	4	1.58±0.24	3.57±0.29
	10	4	1.33±0.36	3.48±0.31
	20	5	1.34±0.26	3.35±0.31

Panaxytriol was intraperitoneally administered for 6 consecutive days.
Carbon clearance was performed 24 hr after the last sample administration by the method of Biozzi.
Each value represents mean ± S.E.

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