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Effect of Ginseng Saponin on the Proliferation and Viability of Murine Thymocyte, *in vitro*

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생쥐 흉선세포의 증식과 생존력에 미치는 인삼 사포닌의 영향

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Abstract

Ginseng saponin had an effect on the proliferation and viability of cultured murine thymocytes.

When the thymocytes were cultured in various concentrations of ginseng saponin, the number of thymocytes increased at 10^{-5}% ginseng saponin but decreased at 10^{-1}%.

There was little change in the number of thymocytes when cultured in IL 2(Interleukin 2), a factor known for its influence on the proliferation and maturation of thymocytes.

When the thymocytes were cultured in various concentrations of IL 2 with 10^{-5}% ginseng saponin, the number of total cells increased at 1.5% or 3% IL 2 when cultured for 9 hours, or at 6% IL 2 for 12, 24, or 48 hours. But there was little change in the number of viable cells.

In vitro, ginseng saponin had an effect on the activity of ADA(Adenosine Deaminase), an enzyme known to affect the production of IL 2. There was a 25% increase in the activity of ADA in the presence of 10^{-5}% ginseng saponin.

Introduction

The thymus is generally considered the major site for the maturation of immunocompetent T lymphocytes[1]. The precusors of T cells in bone marrow are migrated to the thymus through the blood, differentiated into mature cells, and transferred to

lymph nodes, spleen and peripheral blood[2-5].

The mature T lymphocytes in peripheral blood and spleen produce Interleukin 2(IL 2) in response to mitogenic or antigenic stimulation. And IL 2 stimulates to proliferate the other T cells, especially cytotoxic T cell line[6-9]. The relative IL 2 production and its activity by thymocytes are much lower than by peripheral T cells[10-12]. And thymocytes are unable to synthesize the effective IL 2 under Adenosine Deaminase (ADA) deficiency conditions. ADA deficiency preferentially affects thymocyte differentiation and proliferation as well[13].

The physiological effects of ginseng saponin have been observed, and the present study was undertaken to investigate the effects of ginseng saponin on the proliferation and viability of murine thymocytes, the activity of IL 2, and the activity of ADA.

Material and Method

Ginseng saponin was prepared from powdered Korean white ginseng roots(Keumsan, 4 years, 50pcs/300g) according to the procedure described in Fig. 1.

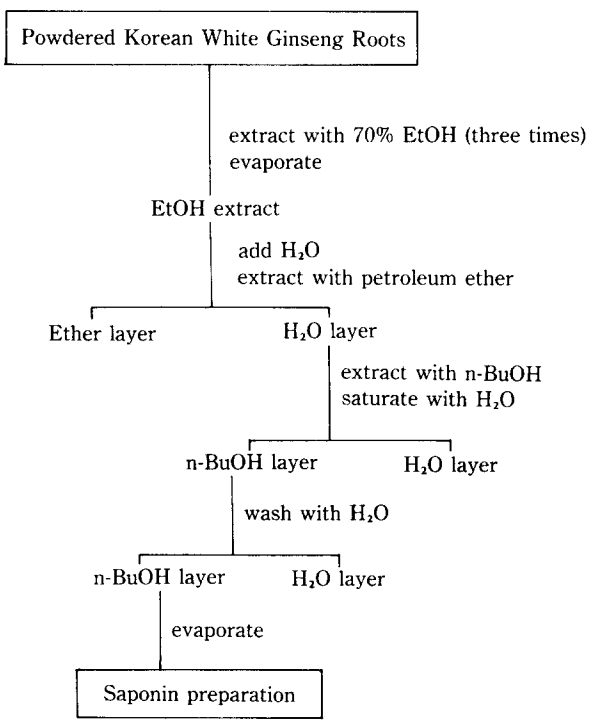

Fig. 1. Extraction procedure of total saponin from the ginseng.

Table 1. Effect of ginseng saponin on the total cells of murine thymocytes *in vitro*.

Number of cells (/ml × 10^{-5})

Time (hr)	Conc. of ginseng saponin(%)						
	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3	98.31±3.44	90.67±6.38	108.77±5.60	106.27±6.33	104.40±4.00	96.60±3.60	86.70±7.61
6	95.64±4.16	86.50±7.52	97.00±11.32	90.35±7.30	97.55±5.39	94.97±7.36	77.07±6.96[a]
9	78.57±6.26	77.75±4.75	95.90±7.04	89.70±4.84	89.75±7.07	84.72±8.31	71.22±12.49
12	80.41±4.20	85.90±14.08	94.82±15.66	84.67±9.22	91.40±7.58	83.62±8.03	66.27±10.34
18	85.45±5.12	88.20±8.42	101.72±11.29	92.12±5.59	101.80±7.33	79.10±9.14	55.37±10.93[a]
24	78.31±3.34	72.85±6.15	96.02±11.59	82.82±3.80	83.27±4.38	78.60±6.66	61.60±11.80
48	79.48±4.09	76.32±9.31	86.92±6.93	89.72±8.80	89.62±9.12	78.60±11.36	37.60±7.15[b]
72	83.06±6.00	79.63±5.08	101.70±3.55[a]	92.96±3.41	96.93±2.86	65.60±11.71	39.50±5.80[b]

The number of cells are expressed as mean±S.E..
a:P<0.05 b:P<0.01.
The cells were obtained from three mice.

IL 2 for use was produced by 24 hr stimulation of ICR mouse spleen cells(1×10^6 cells/ml) with Con A(2.5ug/ml). Cells were cultured in humidified atmosphere of 5% CO_2 in air and removed by two sequential centrifugations (2000g for 10 min and 10,000g for 20 min). The supernate was used for the thymocyte cultures and assayed for the presence of IL 2 activity using Sephadex G-100 column chromatography(1.5×90cm)[9-14].

For thymocyte cultures, 2 to 4-week-old ICR mice were used in all experiments. Each thymocyte was aseptically removed and teased apart with forceps in HBSS. The resulting cell suspension was allowed to sediment for 5 min at 300g to remove cell clumps. After washing, the cells were resuspended at a cell concentration of 1×10^7 cells/ml and cultured in Dulbecco's Modified Eagles medium containing 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37°C. Numbers of viable and dead nucleated cells were counted in a hemocytometer after addition of 1% trypan blue[15-16].

ADA was assayed by method of Barton et al.[17].

Results and Discussion

It was studied that the effect of ginseng saponin on cell division tends to proportional to the concentration of it by optimal dose and inversely proportional to it beyond optimal dose[18]. When the thymocytes were cultured in various concentrations of ginseng saponin, the number of total thymocytes were increased significantly at 10^{-5}% ($P<0.05$) for 72 hr culture but not significantly for the other hours(Table 1). They were decreased significantly at 10^{-1}% for 6($P<0.05$), 18($P<0.05$), 48($P<0.01$), and 72($P<0.01$) hr cultures. And it was at 10^{-5}% that the number of viable cells were augumented

significantly for 12($P<0.05$) and 72($P<0.01$) hr cultures (Table 2). At 10^{-1}% they were decreased significantly for 6($P<0.05$), 12($P<0.01$), 18($P<0.05$), 48($P<0.05$), and 72($P<0.05$) hrs. The number of viable and total cells were similar or increased slightly at the other concentrations. Therefore it could be inferred from these results that the optimal dose of ginseng saponin on the proliferation of thymocytes seem to be 10^{-5}%. There were many papers about its mechanisms. Some of these were that the extracts of Panax ginseng mostly facilitates cellular synthesis of nucleic acids in various organs[19], that it accelerates DNA, protein, and lipid synthesis on mitosis in rat bone marrow[20], and that it enhances DNA synthesis by shortening DNA synthetic period and mitotic cell cycle[21]. It seems to be due to these mechanisms in our results.

Table 2. Effect of ginseng saponin on the viability of murine thymocytes, *in vitro*

Number of cells (/ml × 10^{-5})

Time (hr)	Conc. of ginseng saponin(%)						
	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3	95.22± 5.60	89.47± 5.81	101.82± 7.94	103.92± 6.38	102.75± 4.86	94.50± 4.29	89.22± 7.87
6	90.14± 5.02	78.82± 8.51	92.92±11.55	83.72± 8.77	91.57± 4.96	89.57± 7.66	68.17± 6.49[a]
9	63.76±10.81	67.50± 5.24	82.12± 7.97	79.35± 5.88	80.32± 8.18	76.52± 8.02	63.97±12.24
12	62.94± 2.22	64.93± 9.57	91.60± 8.73[a]	69.12±10.14	75.15± 8.11	69.97± 8.70	40.17± 2.58[b]
18	52.18± 6.15	53.50± 8.21	62.77± 7.26	69.10± 8.55	67.55± 7.32	54.72± 8.02	28.30± 6.76[a]
24	36.27± 3.78	35.70± 4.07	47.42± 5.45	39.57± 7.01	36.35± 4.01	37.52± 0.90	27.37± 6.36
48	18.32± 2.12	17.55± 3.58	21.60± 3.85	20.15± 2.57	22.55± 2.77	14.47± 0.94	10.93± 2.46[a]
72	11.28± 1.24	10.40± 1.55	20.76± 2.08[b]	16.33± 2.46	10.33± 1.22	7.46± 0.67[a]	5.73± 1.21[a]

The number of cells are expressed as mean±S.E..
a:$P<0.05$ b:$P<0.01$.
The cells were obtained from three mice.

In the various concentrations of IL 2 the number of viable and total cells were similar to the control (Tables 3 and 4). It has been known that mitogenesis does not depend upon mitogen or antigen directly but upon a lymphokine(IL 2) released by mature T cells in response to mitogen or antigen[7]. IL 2 interacts with the IL 2 receptors which is induced to express by mitogen or antigen, thus causing mitogenesis[22-24]. And it shows a direct mitogenic effect on spleen cells and lymph nodes but not on unfractionated thymocytes[25]. These reports were consistent with our results. The reason has not been clear yet.

Being cultured in various concentrations of IL 2 with 10^{-5}% of ginseng saponin to observe the effect of ginseng saponin on IL 2, the total cells were increased at 1.5,

Table 3. Effect of IL 2 on the total cells of murine thymocytes, *in vitro*

Number of cells (/ml × 10^{-5})

Time (hr)	Conc. of ginseng saponin(%)													
	Control		1.5		3		6		12.5		25		50	
0	100.00		100.00		100.00		100.00		100.00		100.00		100.00	
3	98.31 ±	3.44	86.80 ±	5.66	101.06 ±	13.54	95.30 ±	9.06	102.00 ±	11.25	103.40 ±	2.51	92.60 ±	8.00
6	95.64 ±	4.16	80.66 ±	7.99	102.56 ±	12.58	85.33 ±	7.08	94.30 ±	4.59	92.50 ±	4.27	102.60 ±	7.60
9	78.57 ±	6.26	80.43 ±	2.96	87.70 ±	4.43	77.03 ±	3.01	88.06 ±	6.71	80.36 ±	3.58	83.43 ±	6.54
12	80.41 ±	4.20	79.23 ±	5.17	103.53 ±	12.13	84.10 ±	9.37	84.60 ±	10.10	94.90 ±	12.44	74.06 ±	4.10
18	85.45 ±	5.12	76.30 ±	6.60	100.95 ±	1.35[a]	82.16 ±	8.68	82.63 ±	13.87	87.20 ±	14.10	84.23 ±	8.02
24	78.31 ±	3.34	69.85 ±	2.95	79.30 ±	5.90	73.95 ±	2.75	86.25 ±	5.75	91.95 ±	2.55[a]	81.23 ±	8.02
48	79.48 ±	4.09	63.93 ±	7.68	77.40 ±	12.32	68.43 ±	6.56	76.26 ±	11.72	75.93 ±	10.33	81.80 ±	4.30
72	83.06 ±	6.00	74.80 ±	5.05	84.50 ±	1.27	71.90 ±	8.32	76.70 ±	9.51	82.86 ±	6.19	75.00 ±	3.50

Table 4. Effect of IL 2 on the viability of murine thymocytes, *in vitro*

Number of cells (/ml × 10^{-5})

Time (hr)	Conc. of ginseng saponin(%)													
	Control		1.5		3		6		12.5		25		50	
0	100.00		100.00		100.00		100.00		100.00		100.00		100.00	
3	95.22 ±	5.60	92.15 ±	1.95	99.35 ±	13.35	102.30 ±	1.40	114.85 ±	3.25[a]	101.80 ±	4.70	96.05 ±	14.70
6	90.14 ±	5.02	77.20 ±	8.10	89.20 ±	1.00	81.90 ±	3.70	88.85 ±	0.45	95.60 ±	4.50	101.65 ±	3.75
9	63.73 ±	10.81	58.03 ±	8.94	60.60 ±	7.89	53.90 ±	6.03	64.10 ±	7.12	56.96 ±	8.52	57.26 ±	12.96
12	62.94 ±	2.22	55.73 ±	7.23	66.06 ±	9.39	57.50 ±	7.82	58.16 ±	3.33	61.66 ±	5.06	64.26 ±	0.96
18	52.18 ±	6.15	46.73 ±	15.86	42.45 ±	3.65	45.83 ±	15.28	45.43 ±	3.90	44.36 ±	2.03	47.70 ±	2.22
24	36.27 ±	3.78	35.80 ±	4.40	33.90 ±	11.00	36.15 ±	0.85	36.35 ±	3.15	38.20 ±	0.93	37.65 ±	7.55
48	18.32 ±	2.12	14.50 ±	0.25	21.33 ±	3.84	22.25 ±	2.65	19.36 ±	1.16	17.70 ±	0.70	19.40 ±	0.80
72	11.28 ±	1.24	21.10 ±	2.80[a]	17.75 ±	5.85	9.40 ±	0.70	11.75 ±	0.75	11.30 ±	0.30	11.75 ±	3.35

3 and 6% of IL 2(Table 5). The viable cells were decreased at 50% for 6 hr culture and no significant changes at the others(Table 6). These could be interpretated that both ginseng saponin and IL 2 which stimulate to proliferate the cells by reducing DNA synthetic period react synergistically, increasing significantly for 9 hr culture or that the low concentrations of IL 2 be activated by ginseng saponin. And we could not exclude the fact that ginseng saponin affects the IL 2 receptor [26]. It requires futher studies to manifest the detailed mechanisms of these.

Table 5. Effect of ginseng saponin[a] and various concentrations of IL 2 on the total cells of murine thymocytes, *in vitro*

Number of cells (/ml × 10^{-5})

Time (hr)	Conc. of IL 2(%)						
	Control	1.5	3	6	12.5	25	50
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3	98.31± 3.44	91.03± 2.83	95.96± 8.65	103.73± 7.32	93.30± 4.02	104.56± 4.02	81.60± 9.03
6	95.64± 4.16	103.43± 1.55	101.90± 10.75	96.46± 7.20	96.03± 0.78	111.30± 3.59[b]	88.80± 6.00
9	78.57± 6.26	94.73± 1.31[b]	99.06± 2.24[b]	93.80± 9.51	93.73± 6.87	94.30± 6.04	92.23± 1.07
12	80.41± 4.20	98.76± 7.17	89.53± 2.93	92.93± 3.17[b]	85.26± 2.69	85.10± 3.67	78.00± 8.27
18	85.45± 5.12	88.90± 4.12	83.16± 5.75	89.86± 10.71	80.40± 11.10	87.60± 2.60	80.20± 5.99
24	78.31± 3.34	82.40± 3.69	90.06± 12.95	98.20± 7.04[b]	93.20± 8.03	79.76± 6.03	80.93± 7.40
48	79.48± 4.09	97.66± 5.61[b]	94.90± 15.72	99.36± 7.42[b]	84.70± 1.64	95.16± 8.21	84.60± 8.60

Table 6. Effect of ginseng saponin[a] and various concentrations of IL 2 on the viability of murine thymocytes, *in vitro*

Number of cells (/ml × 10^{-5})

Time (hr)	Conc. of IL 2(%)						
	Control	1.5	3	6	12.5	25	50
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3	95.22± 5.60	85.13± 0.93	94.30± 8.72	99.30± 10.45	88.03± 6.36	101.13± 5.32	81.00± 6.25
6	90.14± 5.02	87.80± 8.67	85.40± 3.21	87.40± 5.02	87.36± 3.15	89.83± 6.61	74.40± 1.03[b]
9	63.76± 10.81	69.96± 4.22	71.10± 5.58	66.86± 14.23	65.96± 6.86	67.10± 9.99	63.16± 6.03
12	62.94± 2.22	67.96± 12.69	59.16± 6.81	55.50± 9.82	51.66± 6.87	45.53± 10.40	65.80± 6.80
18	52.18± 6.15	43.86± 2.57	40.56± 3.59	47.65± 10.85	36.10± 5.34	42.06± 2.01	37.60± 5.50
24	36.93± 3.11	45.50± 12.40	37.96± 1.56	31.96± 1.90	38.50± 0.20	37.00± 0.13	37.66± 4.69
48	18.32± 2.12	23.53± 7.22	19.90± 7.67	12.73± 1.42	15.73± 3.49	23.36± 9.62	24.25± 7.65

a: The concentration of ginseng saponin is 10^{-5}%.
b:$P<0.05$
The number of cells are expressed as mean ± S.E..
The cells were obtained from three mice.

Finally, as shown in Fig. 2 there was a 25% increase in the activity of ADA at 10^{-5}% of ginseng saponin and little change at the other concentrations. These could be explained by that the optimal concentration is needed for the activation of enzyme, that it is different from each enzyme [27], and that it activates the enzyme by lowering the

Km of substrate or by altering Km due to the conformational change of enzyme[28].

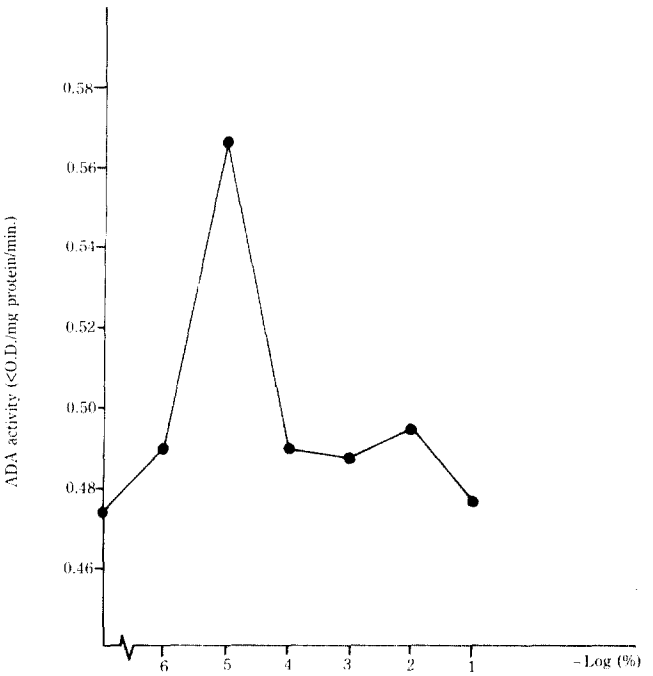

Fig. 2. Effect of ginseng saponin on the ADA activity of murine thymocytes, *in vitro*.

요 약

면역 작용에 중요한 역할을 하는 림프구의 일종인 T 세포가 성장 및 증식하는 기관인 흉선의 흉선세포증식과 생존력에 미치는 인삼 saponin 의 영향과, 흉선 세포의 증식에 관여하는 Interleukin2(IL2)에 미치는 인삼 saponin 의 영향과, IL2의 생성에 영향을 미치는 Adenosine Deaminase(ADA)에 대한 인삼 saponin 의 영향 등을 알아보기 위하여 생쥐의 흉선세포를 사용하여 다음과 같은 결과를 얻었다.

여러 농도의 인삼 saponin 에서 배양한 전체 흉선세포수와 살아있는 흉선세포수의 변화는 10^{-5}% 농도에서 대조군보다 증가하였으나 10^{-1}% 농도군에서는 감소되었다.

여러 농도의 IL2에 배양한 결과는 대조군과 별 차이가 없었다.

여러 농도의 IL2에 인삼 saponin 10^{-5}% 농도를 첨가하여 배양한 전체 흉선세포수는 1.5%, 3% 및 6%농도군에서 9시간 또는 12시간배양시 증가하였으며, 6% 실험군은 24시간과 48시간 배양시에도 유의성있게 증가하였다. 그러나 살아있는 흉선세포수는 대조군

과 별 차이가 없었다.

마지막으로 흉선세포의 ADA 활성도에 미치는 인삼 saponin의 영향은 10^{-5}%농도에서 25%의 유의성있는 활성 증가가 일어났다.

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