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Effect of Ginsenosides on Liver Cell Proliferation in Relation to Its Clinical Applicability

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Abstract

We have studied the ginsenoside effect on DNA, RNA and protein synthesis in bone marrow. Ginseng was found to have a beneficial effect on α-napthyl-isothio-cyanate-induced hepatobiliary damage. Stimulatory effects of some ginsenosides were shown for cell proliferation and DNA synthesis in a culture liver cell line. The clinical applicability of ginseng on liver diseases will be discussed in relation to traditional medicine.

Introduction

In the 1st Int'l Ginseng Symposium, we reported that ginsenosides, ginseng saponins, had a stimulatory action on DNA synthesis in bone marrow.[1] Hepatic RNA and protein synthesis stimulatory action of ginseng has been reported by Oura et al. over 20 years before[2]. Also, CCl_4-induced liver damage was restored with administration of ginseng saponins according to Hahn.[3] We have already reported that α-naphthyl isothiocyanate-induced hepatobiliary damage was reduced by ginseng saponin and ginseng.[4]

In this paper, we investigated effect of ginsenosides on liver cell proliferation together with effect on DNA synthesis in the cell culture system. Through these experimental studies, we tried to know a possible significance of ginseng in clinical therapeutics as to hepatic diseases.

Materials and methods

Male rats of Wistar strain, 7 week-old, received the *in situ* perfusion of the liver with collagenase solution through portal vein according to the method of Nakamura and Ichihara[5]. The suspension of isolated dispersed liver cells in 0.5 ml of medium L-15 thus obtained, was placed on every collagen precoated 24 wells of a multiplate, with which the primary monolayer culture of liver cells was performed. The cells were put into each well in 0.5ml of Dulbecco's minimum essential medium containing 0.5 ng/ml of insulin with some of epidermal growth factor (EGF) (1-5ng/ml), newborn calf serum (NCS) (4-20%) or ginsenosides (5ug/ml). The cell culture of hepatocytes was performed using CO_2 incubater under 10% CO_2 -90% O_2 at 37°C for 2 to 9 days.

DNA synthesis in cultured cells was studied using [^{3}H] methyl-thymidine (NEN, specific activity : 2.0 Ci/mmol). After 6 hours of cell culture, cells were washed with new medium and subjected to the radioactivity determination with a liquid scintillation counting system (Aloka LSC-900) in Tarkkanen's solution[6].

Results

Change of cell counts of cultured liver cells : Primary cell culture of hepatocyte was performed for up to 9 days. Cell numbers in each well were counted with an aid of cell counter scale. Effect of NCS, EGF and/or ginsenoside Rb_2 on number of cells at the incubation period of 2 days, was studied (Table 1).

All of EGF, NCS and ginsenosides had stimulatory action on cell proliferation. The same tendency was observed at the period of 9 days(Table 2).

The comparison between various ginsenosides as to increasing action on cell number was also done.

Among ginsenosides, Rb_1, Rb_2, Rc and Rg_1 were relatively potent at the period of 2 days (Table 3) and almost all ginsenosides tested were found to be effective at the period of 5-9 days(Table 4, 5).

Table 1. Effect of ginsenoside on liver cell proliferation in relation to EGF and calf serum

Caif serum (−) :	
No addition	1.0
EGF, 1 ng/ml	1.2
Ginsenoside Rb_2, 5 ug/ml	1.3
EGF + Rb_2	1.4
Calf serum, 10%(+) :	
No addition	1.1
EGF, 1 ng/ml	1.4
Ginsenoside Rb_2, 5 ug/ml	1.3
EGF + Rb_2	1.5

37°C, 48 hours, viable cells, n=4

Table 2. Effect of ginsenosides on liver cell proliferation in relation to EGF and calf serum

	Cell count ($\times 10^5$/well)	
Calf serum (−) :		
No addition	2.2	(100) %
EGF	2.1	(95)
Ginsenoside Rb_2	2.8	(127)
EGF & Rb_2	3.5	(159)
Calf serum, 10%(+) :		
No addition	2.5	(114)
EGF	3.7	(168)
Ginsenoside Rb_2	4.2	(191)
EGF & Rb_2	3.7	(168)

Incubation: 9 days, 37°C

Table 3. Effect of ginsenosides on proliferation of cultured liver cells

Ginsenosides	Cell count ($\times 10^5$/well)	
No addition	1.1± 0.1*	
Ro	1.3± 0.2	N.S
Rb_1	1.6± 0.2	$p<0.05$
Rb_2	1.7± 0.2	$p<0.05$
Rc	1.6± 0.2	$p<0.05$
Rd	1.3± 0.2	N.S
Re	1.4± 0.2	N.S
Rg_1	1.6± 0.2	$p<0.05$

37°C, 48 hours, viable cells
* Mean ± S.E., n=6
Newborn calf serum, 10%
EGF, 1 ng/ml

Table 4. Effect of ginsenosides on proliferation of cultured liver cells (5 day incubation)

Ginsenosides	Cell count ($\times 10^5$/well)	
No addition	1.3	(100) %
Ro	2.0	(154)
Rb_1	2.6	(200)
Rb_2	2.8	(215)
Rc	3.3	(254)
Rd	2.3	(177)
Re	3.0	(231)
Rg_1	3.0	(231)

Table 5. Effect of ginsenosides on proliferation of cultured liver cells (9 day incubation)

Ginsenosides	Cell count ($\times 10^5$/well)	
No addition	1.7	(100) %
Ro	1.8	(106)
Rb_1	4.0	(235)
Rb_2	5.2	(306)
Rc	5.8	(341)
Rd	5.8	(341)
Re	2.5	(147)
Rg_1	3.0	(176)

Table 6. Effect of serum and EGF on DNA synthesis in cultured liver cells

EGF	Serum (16%)		Serum	EGF(4ng/ml)	
ng/ml	+	−	%	+	−
0	28	16	0	21	17
1	47	11	4	26	20
2	47	15	8	21	19
3	64	13	12	30	23
4	36	17	16	23	25
5	37	13	20	15	32

DNA synthesis x10dpm

Table 7. Effect of Ginsenosides on DNA synthesis in cultured liver cells

Ginsenosides	DNA synthesis EGF (3ng/ml) +		x10dpm −	
No addition	21	(100) %	14	(100) %
Ro	22	(105)		−
Rb_1	20	(95)	16	(114)
Rb_2	33	(157)	22	(157)
Rc	23	(110)	16	(114)
Rd	21	(100)		−
Re	18	(86)	22	(157)
Rg_1	42	(200)	27	(193)

Serum, 10%

The maximum cell numbers were attained at 9 day incubation with diol group of ginsenosides such as Rb_1, Rb_2, Rc or Rd, while no further increase was observed after 5 days with triol group of ginsenoside Re or Rg_1. These data revealed that some of ginsenosides had a promoting action of cell proliferation. Change of DNA synthesis in cultured hepatocytes.

After determining the optimal concentrations of EGF and NCS(Table 6), [^{3}H] thymidine incorporation into hepatocytes were determined with various ginsenosides(Table 7).

Ginsenosides Rb_2 and Rg_1 were found to be potent in stimulation of DNA synthesis this time. Effect on cell proliferation seemed to be consistent with that on DNA synthesis.

Discussion and Summary

In this paper, stimulatory effects of ginsenosides on cell proliferation and DNA synthesis were reported. It was reported that hepatocyte mitosis was initiated by low populated cell culture system containing serum, EGF and insulin[7]. Ginsenosides seems to be potent as comparable to EGF or serum and had an additive action to EGF of serum.

References

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임상적 적용과 관련된 간세포 증식에 미치는 Ginsenosides 의 효과

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골수에서 DNA, RNA 및 단백질에 대한 인삼의 효능을 조사하였던 바 α-naphthyl-isothio-cyanate 로 유도된 간담즙성 상해에도 좋은 효과가 있을 것으로 사료 되었다. 본 연구결과 몇가지 인삼성분이 배양한 간세포에서 DNA합성과 세포증식에 촉진효과를 나타내었다. 또한 전통적인 치료제와 관련하여 간질환에 대한 인삼의 임상적 효과에 대해서 논의하고자 한다.