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Protective Effect of Ginseng Polysaccharide Fraction on CCl_4-induced Hepatotoxicity *in vitro* and *in vivo*

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Abstract□Effect of ginseng polysaccharide fraction was examined for CCl_4-induced hepatotoxicity *in vitro* and *in vivo*. In CCl_4-injured primary cultured rat hepatocytes, treatment of the polysaccharide fraction (0.1, 0.3, 1.0 mg/m*l*) significantly inhibited the release of LDH and GOT into the culture medium in a dose-dependent manner. Oral administration of the polysaccharide fraction (100, 200 mg/kg) inhibited the decrease of body weight and the increase of the ratio of liver to body weight in CCl_4-intoxicated rats. Elevation of GOT, GPT and ALP activity in the serum by CCl_4-induced hepatotoxicity was suppressed by administration of ginseng polysaccharide fraction. MDA levels increased in the serum as well as in the liver tissue by treatment with CCl_4 showed a tendency to be low in the rats given to the polysaccharide fraction. These results suggest that the polysaccharide fraction may be active substance responsible for antihepatotoxic effect of *Panax ginseng*.

Key words□*Panax ginseng*, polysaccharides, CCl_4, antihepatotoxicity, primary cultured rat hepatocytes.

Introduction

It have been recognized that total saponin, ginsenosides, and polyacetylenes from *Panax ginseng* showed protective effects on hepatotoxicity induced by various hepatotoxins such as CCl_4, D-galactosamine and thioacetamide in animals as well as primary cultured rat hepatocytes.[1-5]

Recently, ginseng polysaccharides have emerged as important substances contributing to various pharmacological effects of *Panax ginseng*. It has been reported that ginseng polysaccharides showed antitumor,[6-8] immunomodulating,[9-11] hypoglycemic[12-15] and anticomplementary activity,[16,17] and to inhibit toxohormone L-induced lipolysis[18] and to improve avoidance behavior related to learning and memory function.[19] Also, anti-ulcer polysaccharide from a weakly acidic polysaccharide fraction of ginseng leaves was purified.[20,21]

It was suggested the possibility that polysaccharides could treat or improve liver diseases such as hepatic cirrhosis and chronic hepatitis. Glucans from the cell wall of *Saccharomyces cerevisiae* were effective for murine viral hepatitis[22] and polysaccarides from Sappan Lignum, Mori Radicis Cortex and Olibanum showed protective effects against CCl_4- and D-galactosamine-induced hepatotoxicity in ICR mice.[23] Glycoprotein from *Ganoderma lucidum*, which has antitumor activity due to the activation of immune system revealed to inhibit hepatic cirrhosis (fibrosis) by bile duct ligation/scission and the liver damage induced by ethanol, CCl_4 or thioacetamide.[24,25]

In this study, protective effect of ginseng polysaccharide fraction on CCl_4-induced cytotoxicity in primary cultured rat hepatocytes and CCl_4-induced hepatotoxicity in rats was tested.

Materials and Methods

1. Animals and experimental design

Male Sprague-Dawley rats, weighing 150~180 g were supplied by our Animal Breeding Laboratory. They were housed in a room at 22± 2℃ with 55± 5

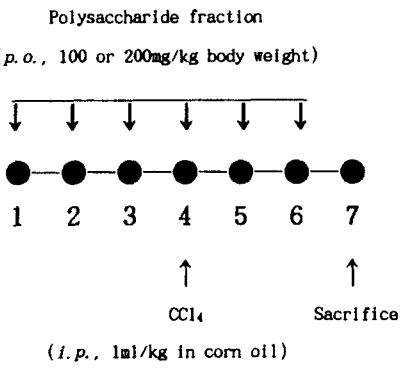

Fig. 1. Experimental design.

% relative humidity and given a defined laboratory rodent chow (Sam Yang Inc. Ltd.) and tap water *ad libitum*.

To assess antihepatotoxic effects of ginseng polysaccharide fraction *in vivo*, experimental schedule was designated as shown in Fig. 1. Rats were orally administered with ginseng polysaccharide fraction at the dose of 100 or 200 mg/kg for 6 consecutive days, injected intraperitoneally with CCl_4 at a dose of 1 m*l*/kg in corn oil 1 hr after sample administration on day 4, and sacrified on day 7.

2. Preparation of ginseng polysaccharide fraction

Polysaccharide fraction was prepared from Korean red ginseng as described previously.[8,9] Briefly, powdered red ginseng was extracted with 85% methanol, and with hot water. The obtained water extract was dialyzed against distalled water, and precipitated with excess ethanol after centrifugation for removal of insoluble materials. The resulting precipitates were dissolved in suitable volume of distalled water, and lyophilized to obtain polysaccharide fracion as a brown powder.

3. Primary culture of rat hepatocytes

Isolation of rat hepatocytes was carried out according to collagenase perfusion method described by Berry and Friend[26] with a slight modification using Ca^{2+}, Mg^{2+}-free Hanks' buffered salt solution (pH 7.4) containing penicillin (100 U/ml), streptomycin (100 μg/m*l*), 15 mM HEPES, 0.225% $NaHCO_3$ and 10^{-7} M insulin as a perfusion buffer.[27] The viability by trypan blue exclusion was over 85% in each cell preparation. One m*l* of cell suspension (2×10^5/m*l*) was plated in the 24 well plate (Falcon, Primaria), and incubated in a humidified incubator at 37℃ in 95% air/5% CO_2. Culture medium used Willam's E medium supplemented with 10% fetal bovine serum, 4 mM L-glutamine, penicillin (100 U/m*l*), streptomycin (100 μg/m*l*), 10^{-6} M dexamethasone and 10^{-7} M insulin.

4. Treatment of CCl_4 and ginseng sample *in vitro*

Isolated hepatocytes were incubated for 2 hr to attach to the plastic surface of culture plate, then carefully removed the medium, and replaced with a fresh volume. Ginseng polysaccharide fraction dissolved in saline (10 μ*l*) and CCl_4 in DMSO (10 μ*l*) were added simultaneously to the medium (1 m*l*), and cultured further for 90 min.

5. Determination of enzyme activities

Lactic dehydrogenase (LDH), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase (ALP) activities in the medium or serum were determined using commercial kits (Asan Pharmaceutic Co.).

6. Lipid peroxidation

Lipid peroxidation in the serum or liver tissue was measured by the formation of the thiobarbituric acid reactive material, malondialdehyde (MDA) using a method of Ohkawa *et al.*[28] 1,1,3,3-Tetramethoxypropane was used as a standard. Protein content was determined by Bradford's method[29] using bovine serum albumin as a standard.

7. Statistical analysis

Statistical significance was evaluated by student's *t*-test.

Results

1. Inhibitory effect on CCl_4-induced cytotoxicity in primary cultured rat hepatocyte

Primary cultured rat hepatocytes were treated with 1.5 mM CCl_4 and ginseng polysaccharide fraction (0.1, 0.3, 1.0 mg/m*l*) simultaneously, and incubated for 90 min (Table 1). Treatment of CCl_4 induced remarkable cytotoxicities against hepatocytes, which increased the release of LDH, GOT and GPT from hepatocytes to 9.3, 5.1 and 2.8 times compared with CCl_4-untreated control, respectively. The polysaccharide fraction inhibited significantly and dose-dependently the release of LDH and GOT increa-

Table 1. Effect of ginseng polysaccharide fracton on CCl_4-induced cytotoxicity in primary cultured rat hepatocytes

Treatment	Dose (mg/ml)	LDH (Wroblewski unit/ml)		GOT (Karmen unit/ml)		GPT (Karmen unit/ml)	
		$CCl_4(-)$	$CCl_4(+)$	$CCl_4(-)$	$CCl_4(+)$	$CCl_4(-)$	$CCl_4(+)$
Control	–	102± 6	950± 38	24± 2	123± 3	11± 3	31± 4
Polysaccharide fraction	0.1	85± 3	865± 57*	24± 1	111± 8	9± 1	29± 3
	0.3	86± 12	799± 57**	24± 2	102± 3**	11± 3	26± 2
	1.0	93± 9	723± 57**	24± 1	101± 7**	10± 2	27± 2

The hepatocytes (2×10^5 cells/ml) were treated with or without 1.5 mM CCl_4 in DMSO (10 μl) and polysaccharide fraction (dissolved in saline) for 1.5 hr simultaneously after initial plating (2 hr). Enzyme activities were determined in the medium. Each value represents mean± S.D. from 3 experiments. Significantly different from each control : *$p<0.05$, **$p<0.01$.

sed by CCl_4-induced cytotoxicity, and decreased the release of GPT from CCl_4-treated hepatocytes but not significantly. In normal hepatocytes, ginseng polysaccharide fraction had no effect on the release of LDH, GOT and GPT up to 1.0 mg/ml.

2. Antihepatotoxicity *in vivo*

As shown in Fig. 2, intraperitoneal injection of CCl_4 to rats (1 ml/kg) droped body weight by 11% and increased the ratio of liver to body weight by 33% as compared with that in the normal group. Administration of ginseng polysaccharide fraction inhibited dose-dependently the loss of body weight and to increase the ratio of liver to body weight in the range of 9~15%.

As shown in Fig. 3 and 4, CCl_4-induced hepatotoxicity elevated GOT, GPT and ALP activity in the serum to 74%, 135% and 30% compared with each enzyme activity in the normal group, respectively. At the dose of the polysaccharide fraction 100 or 200 mg/kg, CCl_4-induced marked elevation of GOT, GPT or ALP activity in the serum was significantly suppressed to 22~24%, 47%, or 27%, respectively.

As shown in Fig. 5, lipid peroxidation levels in serum and liver tissue were increased by treatment with CCl_4 to 31% and 73%, respectively and were lower in the ginseng-treated rats than only CCl_4-treated rats but not significant.

In these results, antihepatotoxic effect of ginseng polysaccharide fraction between tested doses (100, 200 mg/kg) was similar.

In the normal rats, higher dose (200 mg/kg) used in this experiment had no effect on body weight, the ratio of liver to body weight, enzyme activities

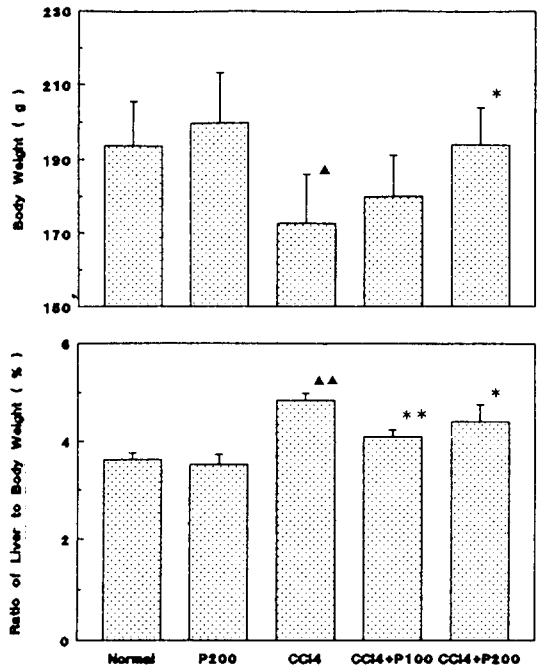

Fig. 2. Effect of ginseng polysaccharide fraction on body weight and liver to body weight in normal or CCl_4-intoxicated rats. Ginseng sample (100, 200 mg/kg) was orally administered from day 1 to day 6. CCl_4 (1 ml/kg in corn oil) was intraperitoneally injected on day 4. Body and liver weights were measured on day 7. Each value represents mean± S.D. for 5 or 6 rats. ▲ : $p<0.05$, ▲▲ : $p<0.01$ vs. normal, *$p<0.05$, **$p<0.01$ vs. CCl_4-treated control.

in the serum as well as MDA contents in the serum and liver tissue (Fig. 2~4), indicating ginseng polysaccharide fraction may be non-toxic *in vivo* up to

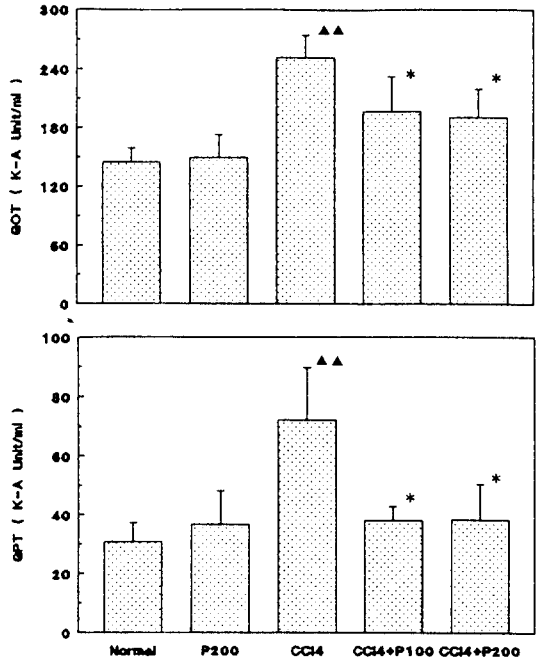

Fig. 3. Effect of ginseng polysaccharide fraction on serum GOT and GPT activity in normal or CCl_4-intoxicated rats. Ginseng sample (100, 200 mg /kg) was orally administered from day 1 to day 6. CCl_4 (1 ml/kg in corn oil) was intraperitoneally injected on day 4. Enzyme activites were determined on day 7. Each value represents mean± S.D. for 5 or 6 rats. ▲▲ : $p<0.01$ vs. normal, *$p<0.05$ vs. CCl_4-treated control.

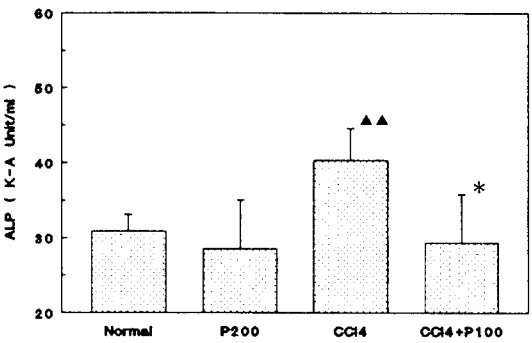

Fig. 4. Effect of ginseng polysaccharide fraction on serum ALP activity in normal or CCl_4-intoxicated rats. Ginseng sample (100, 200 mg/kg) was orally administered from day 1 to day 6. CCl_4 (1 ml/kg in corn oil) was intraperitoneally injected on day 4. Enzyme activites were determined on day 7. Each value represents mean± S. D. for 5 or 6 rats. ▲▲ : $p<0.01$ vs. normal, *$p<0.05$ vs. CCl_4-treated control.

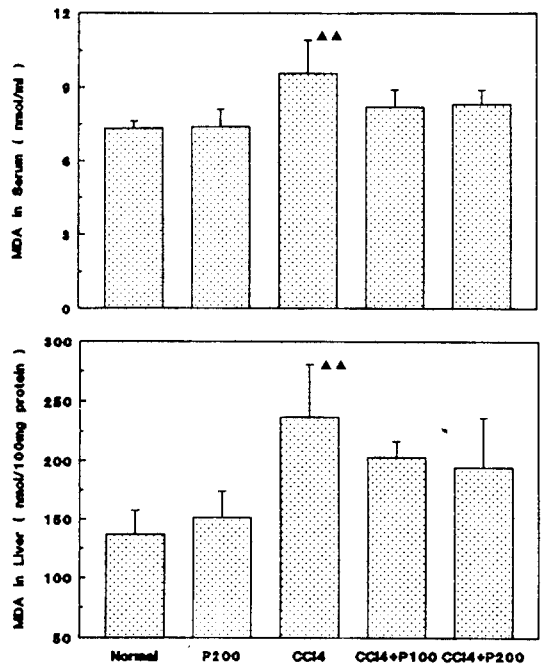

Fig. 5. Effect of ginseng polysaccharide fraction on MDA content in normal or CCl_4-intoxicated rats. Ginseng sample (100, 200 mg/kg) was orally administered from day 1 to day 6. CCl_4 (1 ml/kg in corn oil) was intraperitoneally injected on day 4. MDA contents were determined on day 7. Each value represents mean± S.D. for 5 or 6 rats. ▲▲ : $p<0.01$ vs. normal.

the dose of 200 mg/kg.

Discussion

Antihepatotoxicity is known as an improtant effect among many pharmacological and physiological effects of *Panax ginseng*. It has been reported that ginseng components, such as saponin, ginsenosides, polyacetylenes revealed that liver protective effects in several experimental models using hepatotoxins.[1-5] In the present study, ginseng polysaccharide fraction was found to have protective effect on CCl_4-induced cytotoxicity in primary cultured rat hepatocytes as well as CCl_4-induced hepatotoxicity in rats, indicating the polysaccharide fraction may be a substance responsible for antihepatotoxic effect of *Panax ginseng*.

Numerous investigations have been performed to

develope effective methods and remedies for protecting liver from damage or for the treatment of liver diseases. However, it seems to be no effective therapeutic agents available for hepatitis and liver chirrosis at present time.

Polysaccharides from *Garnoderma lucidum*[24,25] *Saccharomyces cerevisiae*,[22] or several higher plants[23] showed protective effects against liver damages induced by several chemicals and murine viral hepatitis or to inhibit experimental hepatic cirrhosis induced by bile duct ligation/scission in rats. Therefore, it is interesting whether ginseng polysaccharide fraction can alter the liver injury induced by other hepatotoxins such as D-galactosamine and thioacetamide or experimental hepatic cirrhosis.

CCl_4-damaged experimental model has been frequently used to study antihepatotoxic substances. CCl_4 is converted to the highly reactive toxic radical, trichloromethyl free radical ($\cdot CCl_3$) by hepatic cytochrome P-450.[30,31] Toxic free radicals cause lipid peroxidation of cellular organelles and covalently bind to microsomal lipids and proteins.[32,33] Hepatotoxicity of CCl_4 appears to depend on their metabolism and subsquent covalent binding to cellular macromolecules. Recently, it was suggested that cytoplasmic Ca^{2+} level increased by metabolites of CCl_4 might be an important intermediate step in pathological processes induced by CCl_4.[32,34]

To explain possible mode of protective action of ginseng polysaccharide fraction against CCl_4-induced hepatotoxicity, further study on hepatic microsomal enzyme system involving CCl_4 metabolism and detoxifying enzyme such as glutathion-S-transferase will be necessary.

요 약

인삼 다당 분획의 간보호 효과를 일차 배양 흰쥐 간세포와 실험동물에서 CCl_4 유발 간독성에 대하여 조사하였다. CCl_4 처리에 의한 일차 배양 흰쥐 간세포로 부터 배지로의 LDH와 GOT의 유리는 인삼 다당분획(0.1, 0.3, 1.0 mg/ml) 처리에 의해 유의성있게 농도의존적으로 억제되었다. 흰쥐에 다당분획의 경구투여(100, 200 mg/kg)는 CCl_4에 의한 체중의 감소와 체중에 대한 간무게비의 증가를 억제하였고 CCl_4 유발 간독성에 의한 혈청 GOT, GPT 및 ALP 활성 증가를 감소시켰다. 혈청과 간조직의 MDA 함량은 인삼 다당분획 투여군에서 CCl_4 단독 처리군에서 보다 낮은 경향이었다. 이상의 결과는 다당분획은 인삼의 간보호 효과에 기여하는 활성 물질임을 제시하였다.

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