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Protective Effect of Korean Red Ginseng against Oxidative Damage by Carbon Tetrachloride in Rat

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Abstract : To investigate the protective effect of Korean red ginseng (RG) against oxidative damage, rats were intoxicated by carbon tetrachloride and liver tissues and blood were taken and analyzed histopathologically and biochemically. Light microscopy of the liver showed that RG prevented the necrosis of hepatocytes remarkably and reduced the change of fat. RG increased the capability for serum to suppress oxygen radical in the generating system. It is suggested that RG enhanced the antioxidative potential of the body against CCl_4, which would prevent the necrosis of hepatocytes *in vivo*.

Key words : ginseng, oxidative damage, carbon tetrachloride, histologic analysis, radical suppressing activity.

Introduction

Living organism is continuously exposed to oxidation damage by environmental factors[1] such as ozone, nitrogen oxides, xenobiotics as well as by oxygen radical produced endogenously via normal mechanism.[2] It has been suggested that the underlying mechanism in the toxicity of various xenobiotics such as carbon tetrachloride, acetoaminophen, adriamycin and alcohol involves free radical mediated oxidation of biomolecules.[1] CCl_4 has been one of the most widely used xenobiotics as an inducer for the hepatotoxicity which involves severe oxidative damage.[3,4]

The protective effect of Korean ginseng against CCl_4 toxicity has been mainly focussed on its recovering activities of liver function.[5~8] Hahn[5] reported that the total glycosides of Korean ginseng significantly decreased the elevated level of GPT in the serum and lowered the delayed bromosulfophthalein retention time in CCl_4-treated rabbit. His continued report[6] showed that ginsenoside Rb_1, Rg_1 and Re remarkably reduced the hepatocellular swelling, hydropic change and necrosis induced by thioacetamide. Recently, antioxidant activity of Korean ginseng against CCl_4-induced lipid peroxidation was reported by Han and Kim *et al.*[9,10] Han *et al.*[9] reported alcohol extract of Korean ginseng reduced the lipid peroxide level in the CCl_4-treated mouse liver. Kim *et al.*[10] reported that some ginseng components (polyacetylenic compounds) protected liver from the CCl_4-induced hepatotoxicity by inhibiting lipid peroxidation *in vivo*.

In the present study, we induced oxidative damage with CCl_4 in rat and estimated protective effect of Korean red ginseng by histopathologic analysis of liver together with biochemical assay of antioxidant activity of serum from the rat.

Materials and Methods

1. Preparation of Korean Red Ginseng Extract

Six-year-old Korean red ginseng (*Panax gin-*

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seng C.A. Meyer) manufactured by Korea Tobacco and Ginseng Corporation was decocted with water for 3 hours and lyophilized to dryness.

2. Reagents

Carbon tetrachloride was purchased from Waco Chemical Co., Japan. Paraffin oil, Nitroblue tetrazolium (NBT) and 1,1,3,3-Tetraethoxypropane were from Fluka Chemical Co., Switzerland. Hematoxylin, Eosin Y, Xanthine and Xanthine oxidase were the products of Sigma Chemical Co., USA.

3. Animal Treatment

Male Sprague Dawley rats (180~200 g b.w.) were divided into two groups: 9 rats for a control group (CCl_4) and 6 rats for a test group (CCl_4 and ginseng). Lyophilized ginseng extract was dissolved in saline and administered at a dose of 3.7 mg/100 g b.w. (p.o.) one hour before CCl_4-treatment in paraffin oil (p.o.). The dose of CCl_4 was 200 μl/100 g body weight. Rats were anesthetized 24 hours after CCl_4-treatment by urethane and blood was taken by cardiac puncture and then livers were removed.

4. Histologic Analysis

Small pieces of livers were fixed in neutral buffered formalin for 24~48 hours and sections of liver tissues were prepared by the general paraffin embedding method for histologic analysis. Sections were stained with hematoxylin and eosin Y.

5. Biochemical Analysis of Serum

The blood was centrifuged for 20 min at 3,000 rpm and serum was separated. The serum was assayed for its antioxidant activity and malondialdehyde (MDA) level. To assay antioxidant activity, superoxide anion radical was generated by xanthine and xanthine oxidase in the presence of NBT by the method described by Yuda *et al.*[11] Serum was added to the system to estimate its suppressing activity of oxygen radical. The incubation mixture consisted of 2.6×10^{-3} M NBT, 0.1 mM xanthine, 0.1 mM EDTA, 5×10^{-3}% bovine serum albumin and 0.1 m*l* serum in 0.05 M carbonate buffer (pH 10.2). Total volume was 2.9 m*l*. The mixture was placed in a water bath at 25℃ for 10 min, added by 0.1 m*l* of xanthine oxidase (0.02 U/m*l*) and incubated for 20 min. 0.1 m*l* of 2 mM $CuCl_2$ was added to stop the reaction and absorbance was measured at 560 nm. The inhibition rate of reduction of NBT was calculated from the difference between absorbances of the reaction mixtures with and without serum. MDA in serum was measured by the method of Ohkawa *et al.*[12] Thirty μl of rat serum was added by 0.5 m*l* 0.1-N HCl, 0.1 m*l* 10% phosphotungstic acid and 0.3 m*l* 1.0% TBA. The mixture was heated at 95℃ for 50 min, cooled and extracted with 2 m*l* *n*-butanol. After centrifugation, small aliquot from *n*-butanol layer was measured for its fluorescence at ex. 515 nm and em. 546 nm. 1,1,3,3-Tetraethoxypropane was used as a standard for MDA determination. The results were expressed as the means±S.D. Difference between mean values was analyzed by Students' t-test.

Results and Discussion

1. Histologic Findings

CCl_4 induced severe necrosis, fat change and hydropic change in the central zone of hepatic lobule 24 hours after CCl_4-treatment (Fig. 1A). The necrosis was reduced remarkably and fat change was also improved by administration of ginseng extract (Fig. 1B). The toxicity of CCl_4 derives from its toxic metabolites. CCl_4 is metabolized by cytochrome P-450 on microsomal membrane and the resulting $CCl_3^{\cdot}$ or $CCl_3OO^{\cdot}$ radical induces lipid peroxidation at or near their generating loci with the membrane damage including the loss of cytochrome P-450 and glucose-6-phosphatase. Consequently, the ability of endoplasmic reticulum to modulate intracellular calcium concentration is lost and the calcium influx follows leading to necrosis and cell death.[13] It

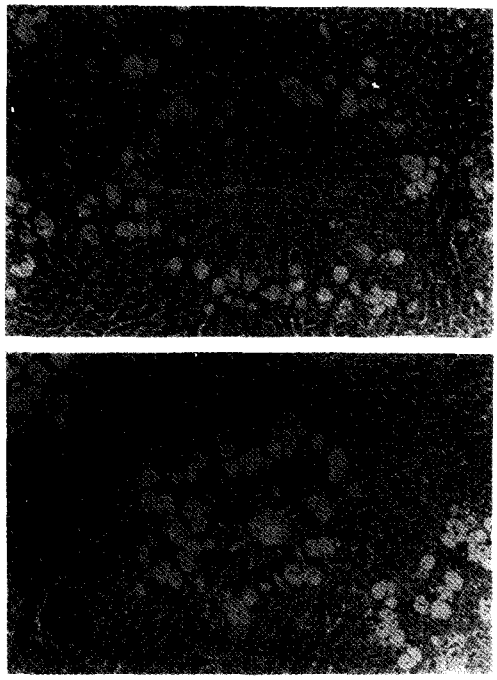

Fig. 1. Photomicrographs of histologic sections of rat liver from the central zone of hepatic lobule. (hematoxylin and eosin Y, ×100). (A) 200 μl CCl_4/100 g b.w. (p.o.). Severe necrosis, fat change and hydropic change in hepatic lobule. (B) 200 μl CCl_4 and 3.7 mg lyophilized Korean red ginseng water extract/100 g b.w. (p.o). Moderate hydropic change of hepatic cells with mild necrosis. Ginseng extract was administered one hour before CCl_4-treatment in paraffin oil (p.o.). Rat liver was removed 24 hours after CCl_4-treatment.

could be postulated that ginseng extract suppressed the generated free radicals or inhibited some CCl_4 metabolizing system which converts CCl_4 to CCl_3 radical.[14]

Regarding that ginseng, which possessed weaker antioxidant activity than other crude drugs containing tannin or flavonoids *in vitro*, showed stronger inhibitory activity against lipid peroxide induction by ethanol in mouse liver than those drugs,[15] the protective effect of ginseng extract against hepatic cellular damage would rather stem from its modulation of CCl_4 metabolism or antioxidant defense system than chemical antioxidant mechanism.

Table 1. Effect of Korean red ginseng on the oxygen radical suppressing activity of serum from CCl_4-treated rat

Treatment	INBT[a] (%)
CCl_4	29.2±6.1
RG[b]+CCl_4	37.6±4.6[c]

Rats were treated with CCl_4 and CCl_4/ginseng. Blood was taken 24 hours after CCl_4-treatment. Values represent means±S.D. (n=6, 9).
[a] Inhibition rate of reduction of nitroblue tetrazolium.
[b] Korean red ginseng water extract.
[c] Significantly different from CCl_4-treated group ($p < 0.01$). Details are described in "Materials and Methods".

2. Oxygen Radical Suppressing Activity of Serum

NBT is a useful detector of superoxide anion radical generated by xanthine/xanthine oxidase system. NBT is reduced by the oxygen radical to form blue formazan pigment which can be monitored at 560 nm. Scavenging or dismutasing of the oxygen radical inhibits the rate of reduction of NBT. We estimated antioxidant activity of rat serum by measuring the inhibition rate of reduction of NBT by added rat serum. The inhibition rate of reduction of NBT was significantly increased by administration of ginseng extract, suggesting that (ginseng+CCl_4)-treated rat serum possessed stronger antioxidant activity than CCl_4-treated one (Table 1).

There are many antioxidants in human body fluids such as haptoglobulin/hemopexin, transferrin, uric acid, α-tocopherol, glucose, bilirubin, albumin and ceruloplasmin.[16] Their mode of actions includes binding free hemoglobin/heme which releases iron readily on exposure to peroxides, binding free iron or copper inhibiting metal-stimulated hydroxyl radical generation, trapping peroxyl and alkoxy radical or reacting with superoxide anion radical and hydrogen peroxide preventing free radical chain reactions.

Our results suggest that Korean red ginseng

Table 2. Effect of Korean red ginseng on the MDA level in the serum from CCl_4-treated rat

Treatment	MDA (nmoles/ml)
CCl_4	4.4±1.1
RG[a]+CCl_4	4.2±0.3

Rats were treated with CCl_4 and CCl_4/ginseng. Blood was taken 24 hours after CCl_4-treatment. Values represent means±S.D. (n=6, 9).

[a] Korean red ginseng water extract. Details are described in "Materials and Methods."

water extract enhance antioxidative potential of the body against CCl_4 by increasing the level of antioxidants which react with oxygen radicals, which would prevent the damage of liver parenchymal cells.

3. Effect on Serum MDA Level

CCl_4 is a powerful pro-oxidant that causes lipid peroxidation. MDA is generated by decomposition of fatty acyl peroxide intermediates derived from membrane phospholipids. We measured MDA level in rat serum to estimate antioxidant activity of ginseng extract. But no effect was found on the level of serum MDA by ginseng (Table 2). MDA is metabolized to CO_2. When rat was intubated with [1,3-^{14}C] MDA, $^{14}CO_2$ production was maximized at 12 hours.[17] MDA has been known to react chemically with biomolecules[18-20] such as nucleic acid, protein and phospholipid. The presence of N-ε-(2-propenal)lysine and N-α-acetyl-ε-(2-propenal)lysine in urine indicates turnover of MDA-modified proteins.[21] Therefore, in our experimental condition (blood was taken after 24 hours after CCl_4-treatment), MDA generated by CCl_4 seemed to be exhausted within the rats, resulting in detection of no effect by ginseng administration. There is a report[10] that increased level of serum MDA from CCl_4-treated rat (35 μl/100 g b.w., i.p.) was lowered by panaxynol, a polyacetylenic ginseng component.

요 약

사염화탄소로 유발되는 산화적 손상에 대한 홍삼의 보호효과를 보기 위하여 사염화탄소 투여 24시간 후 흰 쥐 간의 병리조직학적 검사 결과 홍삼의 물추출물은 간세포의 괴사를 현저히 저해하는 효과를 나타내었다. 한편 홍삼투여군은 대조군에 비해 혈청의 과산화지질 함량을 유의하게 감소시키지 않았으나 혈청의 산소라디칼 소거활성을 유의하게 증가시켰다. 이러한 결과는 홍삼성분이 생체의 항산화활성을 증가시키고 간세포의 괴사를 억제함으로써 생체의 방어기능을 증진시키고 있음을 시사한다.

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