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A Comparative Biological Study of the Rhizome and Main Root from Red and White Ginsengs

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Abstract : Comparative biological activities of 70% methanol extracts from the main roots and rhizomes of both red and white ginsengs were investigated using several *in vitro* experimental models. The main root of red ginseng and the rhizome of white ginseng strongly inhibited lipid peroxidation of hepatic microsomes induced by the non-enzymatic Fe^{2+} / Ascorbate system. The main root and rhizome of red ginseng markedly inhibited the release of GOT, GPT and LDH by CCl_4-induced cytotoxicity in primary cultured rat hepatocytes as compared with those of white ginseng. And also, the main root of red ginseng showed a slight differentiating activity on HL-60 cancer cell line. The results suggest that the rhizome of ginseng have potential as a source of medicinal crude drug with possible pharmacological applications.

Key words : *Panax ginseng*, red ginseng, white ginseng, main root, rhizome, biological activities.

Introduction

The root of ginseng has been traditionally used as a precious medicine in Oriental countries, such as Korea, China and Japan for more than 5,000 years. The root of *Panax ginseng* is steamed and dried to prepare red ginseng, while the peeled roots dried without steaming are designated as white ginseng. The commercially available ginseng roots are classified into two forms, red and white ginseng. Ginseng saponins isolated from the root have been reported to be main effective ingredients responsible for the pharmacological activities, while the rhizome of ginseng has been known to have emetic activity in oriental medicine.[1] Therefore, old literature shows that ginseng has so far been prescribed in the processing of eliminating the rhizome to reduce toxicity and side effects.[2] There are many reports on the comparative pharmacological, chemical and biochemical studies between red and white ginseng.[3] However, little has been known about the comparative biological studies between the main root and rhizome of red and white ginseng. On the other hand, it is well known that the saponin contents were high in the order of fine root, epidermis, rhizome, branch root, pith and cortex, suggesting the possibility of medicinal resource development of the rhizome as well as the leaves.[4,5] Recently, Zhao[6] reported that ginseng rhizome saponin possessed antisenilitive effect and marked effect on relieving the symptom of aging, adjusting organic metabolism and improving physiological functions. A recent report on the biological activity and chemical composition of the rhizome prompted our interest in the comparative investigation of the parts from both white and red ginseng. The purpose of present investigation was ,therefore, to study the comparative biological effects of 70% methanol ex-

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tract between the rhizome and main root of both white and red ginseng.

Materials and Methods

1. Preparation of Ginseng Extracts

Each fifty gram of the rhizome and main root of both Korean red ginsengs (6 year-old steamed and dried ginseng) manufactured by Korea Tobacco and Ginseng Corporation and Korean white ginseng (4 year-old dried ginseng) was extracted with 70% methanol refluxing at 75℃ 4 times. The resulting aliquots were combined, evaporated to dryness and dissolved in 50 m*l* distilled water, which were used for several biological assays.

2. Determination of Saponin by HPLC

Saponin fraction was prepared from the 70% methanol extract by using water-saturated butanol and ginsenosides were measured by HPLC.[7]

3. Effects of Ginseng Extracts on CCl_4-Induced Cytotoxicity in Primary Cultured Rat Hepatocytes

Hepatocytes were isolated from male Sprague Dawley rats (150~200 g) by a collagenase perfusion method based on Berry *et al.*[8] After preincubation for 2 hours, 10 μ*l* of CCl_4 dissolved in dimethylsulfoxide was added to 1 m*l* of fresh culture medium at a concentration of 1.5 mM, followed by incubation for 1.5 hours with or without adding ginseng extracts. Finally, activities of lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were assayed from the medium.[9]

4. Effects of Ginseng Extracts on Rat Hepatic Microsomal Lipid Peroxidation *In Vitro*

Liver was isolated from male Sprague Dawley rat, weighing 180~200 g. Minced liver was homogenized and centrifuged at 12,000×g for 20 min. The resulting supernatant was centrifuged at 100,000×g for 1 hour to pellet microsomes. Microsomal protein was incubated for 1 hour at 37℃ in 50 mM Tris-HCl buffer (pH 7.5) with 10 μM $FeSO_4$ and 0.1 mM ascorbate with or without 1 mg/m*l* of 70% methanol extracts of red and white ginseng. Protein concentration was 1 mg/m*l* in 2 m*l* reaction mixture. The amount of malondialdehyde was measured by TBA method at 533 nm.[10] Inhibition rate against lipid peroxidation was calculated from the decreased absorbance over that of control.

5. Effect of Ginseng Extracts on Differentiation of the HL-60 Cell Line

The HL-60 cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum as described by Breitman *et al.*[11] Cells were plated at a density of 1×10^6 cells/m*l* onto a 96 well microplate. Ginseng extracts were added at a concentration of 0.5 mg/m*l* and diluted 3 times at each step until $(1/3)^6$. As a positive control, retinoic acid dissolved in 95% ethanol was added at a final concentration of 1 μM. Cells were incubated at 37℃ in a humidified atmosphere of 5% CO_2 in the air for 96 hours. Cells were stained for one hour at 37℃ with an equal volume of nitro blue tetrazolium (1 mg/m*l*) dissolved in phosphate-buffered saline containing phorbol 12, 13-dibutyrate (1 μg/m*l*). Morphological observation was performed under light microscopy (×600), and purple-stained and morphologically changed cells were determined to be differentiated.

Results and Discussion

As a part of the studies on the various biological activities of ginseng, the comparative biological test on the main root and rhizome of both red and white ginseng was basically undertaken making use of in vitro bioassays and was focussed on lipid peroxidation, hepatotoxicity and differentiation of cancer cell lines. Since many researchers have recently reported the antiaging

Table 1. Comparison of ginsenoside contents from the main roots and rhizomes of both red and white ginseng (Unit : w/w %)

Samples	Ginsenosides							Total saponin	Crude saponin
	Rb_1	Rb_2	Rc	Rd	Re	Rf	Rg_1		
MRG[b]	0.63[a]	0.42	0.37	0.14	0.27	0.12	0.36	2.31	4.16
RRG	1.62	0.65	0.74	0.34	0.75	0.24	0.68	5.02	8.73
MWG	0.58	0.39	0.33	0.12	0.31	0.13	0.33	2.19	3.93
RWG	1.60	0.61	0.73	0.37	0.73	0.22	0.66	4.93	8.55

[a] Each value was obtained from average of duplicate experiments.
[b] MRG : Main root of red ginseng, RRG : Rhizome of red ginseng, MWG : Main root of white ginseng, RWG : Rhizome of white ginseng.

effects, the protective effects against hepatotoxicity and the anticancer effects of ginseng.

1. Comparison of Ginsenoside Contents from the Rhizome and Main Root of Both Red and White Ginseng

Contents of the major saponins from the main root of red ginseng (MRG), the rhizome of red ginseng (RRG), the main root of white ginseng (MWG) and the rhizome of white ginseng (RWG) were determined by HPLC and the results are shown in Table 1. The saponin contents in the rhizome of both red and white ginseng were found to be relatively higher than those in the main root. But no differences in the saponin composition were observed between main roots and also between the rhizomes, suggesting that other minor ginsenosides may be mainly involved in the differences of biological activities. It is noteworthy that the content of ginsenoside Rb_1 in the rhizome of ginseng is about three times higher than that in main root.

2. Inhibitory Activities of Ginseng Extracts Against Lipid Peroxidation of Hepatic Microsomes Induced by the Non-Enzymatic Fe^{2+}/ Ascorbate System

Table 2 shows the inhibitory activities of the main root and rhizome from both the red and white ginseng against microsomal lipid peroxidation induced by the non-enzymatic Fe^{2+}/Ascorbate system. Free radical mediated damage of unsaturated fatty acids and other cellular components in various biomembranes can result in cumulative degeneration of cellular function, which expresses itself as lipid peroxidation.[12] In this non-enzymatic system, Fe^{2+} catalyzed peroxidative reactions are strongly powerful and capable of oxidizing various compounds including fatty acids, phospholipids, pyridine nucleotide and nucleic acids.[13]

Table 2. Inhibitory activities of ginseng extracts against lipid peroxidation of hepatic microsomes induced by the non-enzymatic Fe^{2+}/Ascorbate

Sample	Concentration	Inhibition rate (%)[c]
MRG[a]	1 mg/m*l*	43.6±8.4[b]
RRG	1 mg/m*l*	27.6±4.3
MWG	1 mg/m*l*	24.0±5.8
RWG	1 mg/m*l*	41.8±6.4

[a] MRG : Main root of red ginseng, RRG : Rhizome of red ginseng, MWG : Main root of white ginseng, RWG : Rhizome of white ginseng.
[b] Each value represents the average±standard error of triplicate experiments.
[c] Lipid peroxide was measured by TBA method (A_{533}).

At the concentration of 1 mg/m*l* of 70% MeOH extract , marked differences of inhibitory effect were observed among the those ginsengs tested. MRG showed 43.6% inhibition, compared with the control, while RRG, 27.6%. In contrast, MWG showed 24.0% inhibition, while RWG did 41.8% inhibition. These results suggest that MRG may be mainly involved in inhibitory activities against microsomal lipid peroxidation

more than that of white ginseng.

3. Inhibitory Effects on CCl_4-Induced Cytotoxicity in Primary Cultured Rat Hepatocytes

A comparison of the inhibitory effects of MRG, RRG, MWG and RWG on CCl_4-induced cytotoxicity in primary cultured rat hepatocytes is shown in Table 3. CCl_4 is known as a typical hepatotoxic agent in liver leading to the dysfunction of several cellular processes and finally to the cell death.[14] When CCl_4 is generally metabolized to its hepatotoxic products by cytochrome P-450, serum transaminases are considered to be released from the liver cells.[15] Hepatocytic treatment with 1.5 mM of CCl_4 for 1.5 hours increased markedly the release of LDH, GOT and GPT 7.3, 4.9 and 2.8 times, respectively, as compared with the controls.

Among those ginseng tested, MRG inhibited the release of LDH, GOT and GPT (28%, 25% and 28% inhibition at the concentration of 0.5 mg/m*l*, respectively), which exhibited a similar trend as was the case for RRG. On the other hand, MWG and RWG exhibited significant inhibitory activities of 23% on the release of LDH, but no significant inhibition was observed on the release of GOT and GPT. In the normal hepatocytes, they had no effect at the concentrations ranging from 0.05 to 0.5 mg/m*l* on the release of these enzymes.

4. Effects of the Main Roots and Rhizome of Ginseng on the Differentiation of HL-60 Cell

The differentiating effects of MRG, RRG, MWG and RWG were measured on the HL-60 cell line and the results are shown in Table 4. Discriminal differences were not found among MRG, RRG, MWG and RWG, however, MRG only showed a slight differentiating activity of 15% at between 56 μg/m*l* and 500 μg/m*l* by comparison with the control group of 10%.

Retinoic acid is a potent inducer of differentiation in numerous established myeloid cell lines as well as primary cultures or cells isolated from patients with promyelocytic leukemia.[16,17] Generally, differentiation-inducing agent is known to show its cytotoxicity,[16] but ginseng was found to show very little.

From the above results, it is very difficult to

Table 3. Effect of ginseng extracts on CCl_4-induced cytotoxicity in primary cultured rat hepatocytes

Sample	Dose (mg/m*l*)	LDH (%)		GOT (%)		GPT (%)	
		CCl_4 (−)	CCl_4 (+)	CCl_4 (−)	CCl_4 (+)	CCl_4 (−)	CCl_4 (+)
Control	-	100±11 (107±11)	100±2 (778±16)[a]	100±6 (33±2)	100±8 (162±13)[b]	100±12 (17±2)	100±5 (47±2)[b]
MRG[c]	0.05	95±2	103± 9	97± 1	89± 1	100± 7	94± 5
	0.1	91±4	95± 7	97± 1	87± 3	100± 5	80± 7[*d]
	0.5	89±3	72± 2[b]	99± 9	75± 3[b]	101±12	62± 5[**]
RRG	0.05	98±10	99± 9	111±12	91± 5	92± 6	105± 5
	0.1	111± 1	98± 8	111± 6	83± 5	99±12	95± 2
	0.5	123± 6	77± 3[**]	107± 3	79± 2[*]	101± 6	84± 5[*]
MWG	0.05	103± 4	106±10	102± 9	106±10	81±15	100±16
	0.1	112± 7	91± 7	101± 3	80±17	82± 2	85±16
	0.5	110± 7	77± 7[*]	113± 2	87±11	78±15	86±11
RWG	0.05	105± 5	95±13	92±17	94± 6	107± 8	82±15
	0.1	100± 9	95± 7	96± 2	91± 3	102± 7	94± 9
	0.5	113± 5	77±10	102± 7	79±15	117±15	84±10

[a] Wroblewski unit/ml, [b] Karmen unit/ml.

[c] MRG : Main root of red ginseng, RRG : Rhizome of red ginseng, MWG : Main root of white ginseng, RWG : Rhizome of white ginseng.

[d] Significantly different from the control: $^*p < 0.05$, $^{**}p < 0.01$.

Table 4. Effects of the main roots and rhizome of ginsengs on differentiation of the HL-60 cell

Sample	Differentiation (%) / Cell growth[c]					
	500 μg/ml	166 μg/ml	56 μg/ml	19 μg/ml	6 μg/ml	2 μg/ml
Control	10/+++	10/+++	10/+++	10/+++	10/+++	10/+++
MRG[a]	15/+	15/++	15/+++	10/+++	10/+++	10/+++
RRG	10/++	10/+++	10/+++	10/+++	10/+++	10/+++
MWG	10/++	10/+++	10/+++	10/+++	10/+++	10/+++
RWG	10/+++	10/+++	10/+++	10/+++	10/+++	10/+++
Retinoic acid[b]	30/+	30/+	30/+	30/+	30/+	30/+

[a] MRG : Main root of red ginseng, RRG : Rhizome of red ginseng, MWG : Main root of white ginseng, RWG : Rhizome of white ginseng.
[b] Retinoic acid was added at a final concentration of 1 μM.
[c] Cell growth was expressed as +++ (confluent), ++ (60~80%, compared to confluent) and + (40~60%, compared to confluent).

give any definite conclusion on the biological differences between main root and rhizome of ginseng, but they may indicate that the rhizome of red ginseng plays partially an important role in the biological and pharmacological activities of ginseng. Further investigation may be needed to confirm comparative biological activities between main root and rhizome of ginseng.

요 약

수종의 *in vitro* 실험 모델을 통하여 홍삼, 백삼의 동체 및 뇌두의 70% MeOH 엑기스에 대한 생물학적 활성 비교를 수행하였다. 홍삼의 동체 및 백삼의 뇌두가 간 microsome에서 비효소적 Fe^{2+} / Ascorbate system 에 의해 유도된 지질과산화를 강하게 억제하였다. 일차 배양 간세포에서 CCl_4에 유도된 간독성의 경우는 홍삼의 동체 및 뇌두가 백삼에 비해 GOT, GPT 및 LDH의 유리를 현저하게 억제하였다. 또한, 홍삼의 동체는 HL-60 암세포에 대한 의미있는 분화 유도를 보여주었다. 이 결과는 인삼의 뇌두도 역시 약용자원으로서의 활용 가능성을 가지고 있다는 것을 시사하고있다.

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