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Ginseng Saponin Prevents the LPS-induced TNF-α Production in Mice

Kyoung Mi Kim, Hye Ju Kim, Jae-Ha Ryu* and Dong Hwan Sohn#

College of Pharmacy and Medicinal Resources Research Center, Wonkwang University, Iksan-City, Geonbuk 570-749, Korea, and

**College of Pharmacy, Sookmyung Women's University, Chungpa-dong, Yongsan-ku, Seoul 140-742, Korea*

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Abstract : Saponins, the major component of ginseng root, mediate the pharmacological action of the ginseng. It has been reported that ginseng roots have protective effect against various toxins. In this study, the effects of ginseng total saponin (GTS) on tumor necrosis factor-alpha (TNF-α) production induced by bacterial toxin was investigated. TNF-α level in lipopolysaccharides (LPS)-activated serum was remarkably reduced by intraperitoneal administration (50 mg/kg) of ginseng total saponin (GTS) into mice. The inhibitory effect against TNF-α production was not significant when GTS was given after the LPS injection, and by oral administration. These results suggested that ginseng root may have protective activity against liver damage accompanying the overproduction of TNF-α and GTS is the active component of ginseng.

Key words : Ginseng, saponin, tumor necrosis factor (TNF-α)

INTRODUCTION

Tumor necrosis factor-alpha (TNF-α) is a peptide originally identified for its ability to cause hemorrhagic necrosis of tumors and cytotoxicity in certain cell lines.[1] It is mainly produced by activated macrophages and participates in a wide range of biological activities.[2-3] TNF-α plays an important role in host defenses in natural cytotoxic cell[4] and macrophages.[5] However, TNF-α cause severe damages such as cachexia, sepsis and hepatic failure.[6-7] A selective inhibitor of the release of TNF-α may, therefore, be beneficial to the treatment of a variety of pathological conditions such as septic shock, rheumatoid arthritis, multiple sclerosis, *etc.* It has been reported that tyrosine kinase inhibitors,[8] bicyclic imidazole,[9] bisbenzylisoquinoline alkaloid[10] and sesquiterpene lactones[11] inhibit the production of TNF-α in LPS-stimulated cells.

Ginseng root is an important remedy in oriental countries, which has been used for thousands of years. Saponins of ginseng root has been known to be the major component which mediate the pharmacological action of the ginseng such as psychotropic effect,[12] changes in energy metabolism,[13] cytotoxicity to various cancer cell lines[14] and antihepatotoxic effect.[15] Some researches have been reported that ginseng saponin has protective effects toward various injury induced by free radicals and toxic chemicals.[16-18] We examined the effect of ginseng saponin on TNF-α production in order to evaluate the protective effect of ginseng root against hepatic injury resulted from the overproduction of TNF-α.

To whom correspondence should be addressed.
(Tel) +82-653-850-6822; (Fax) +82-653-854-6038
(E-mail) dhsohn@wonnms.wonkwang.ac.kr

MATERIALS AND METHODS

1. Preparation of crude ginseng saponin

The powder of Korean ginseng (*Panax ginseng*) root (1 kg) was extracted with methanol with reflux. The methanol extract was partitioned between ether and water to remove a lipid soluble fraction. The water layer was partitioned again between butanol and water. The butanol phase was taken and evaporated under vacuum to yield ginseng total saponin (GTS, 78 g).

2. Effects of ginseng saponin on the level of serum TNF-α

Male ICR mice (25~30 g) were used for the experiment. Lipopolysaccharide (LPS, 2 mg/kg) was administered with or without GTS. GTS were given by intraperitoneal (i.p.) or oral (p.o.) injection. LPS and dexamethasone (DEXA, 2 mg/kg) were administered with intraperitoneal injection.

After 2 h of LPS administration, blood was collected from the carotid artery. After overnight storage of whole blood at 4°C, serum was separated and kept at -70°C for TNF-α assay.

3. ELISA for TNF-α

Immunoreactive TNF-α was assayed by indirect sandwich ELISA. In brief, microtiter plates were coated with monoclonal anti-murine antibody to TNF-α (Genzyme Co., Cambridge, MA), and then samples were added to each well and followed by polyclonal anti-murine TNF-α antibody (Genzyme Co.). After binding of alkaline phosphatase conjugated anti-rabbit IgG (Gibco BRL, Gaithersburg, MD), p-nitrophenylphosphonate was added as a substrate. Optical density was measured at 405 nm in ELISA reader.

4. Statistical analysis

Results were expressed as means ± SD with n indicating the number of mice. Statistical significance of the data was determined by one-way analysis of variance. Mean values from different treatments were compared by Tukeys multiple comparison test where a value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

TNF-α has been shown to be a principal cytokine in the pathophysiology of acute liver injury of diverse origin and the elevation of TNF-α in acute liver injury has been reported.[19] Therefore, the regulation of TNF-α is very important for the treatment of many diseases. During thousands of years, ginseng has been used in oriental countries and the pharmacological actions of ginseng have been re-evaluated by modern scientific manner. Recent researches reported that ginseng saponin has a protective effect of liver damage by toxic chemicals.[20]

We have investigated that the effect of ginseng total saponin (GTS) on the serum TNF-α level in mice. When GTS was coadministered intraperitoneally with LPS into mice, TNF-α level in serum was reduced as shown in Fig. 1. With 50 mg/kg of GTS, the serum TNF-α level was as low as that of normal mouse. The inhibitory effect of GTS on LPS-induced production of TNF-α was not significant with less than 50 mg/kg of GTS. When GTS was administered without LPS, there was no difference in serum TNF-α level between control and GTS-treated mice (data not shown). Dexamethasone, a well-known inhibitor of

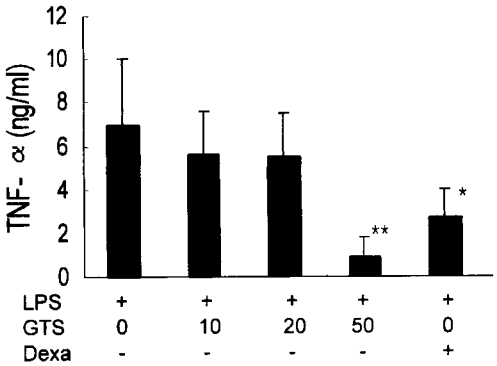

Fig. 1. Effect of ginseng saponin on the serum TNF-α level of LPS-activated mice. LPS (2 mg/kg) was administered by intraperitoneal injection with ginseng total saponin (GTS) or dexamethasone (DEXA, 2 mg/kg). Blood was collected at 2 h after LPS injection and serum was separated for TNF-α assay. Results are represented as the mean ± SD (n=6-8). **p<0.001, *p<0.05, Significantly different from LPS-treated group.

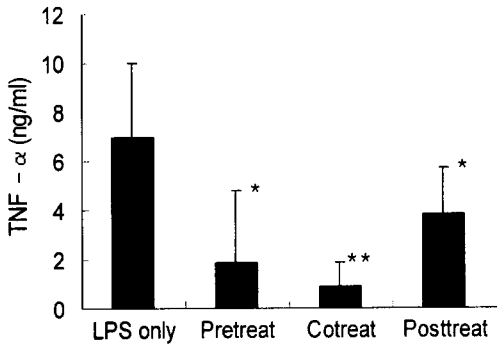

Fig. 2. Effect of dosing time of ginseng saponin on the production of TNF-α Ginseng total saponin (GTS, 50 mg/kg) was intraperitoneally administered with LPS (cotreat), 3 hr before (pretreat) or 30 min after LPS (posttreat). Blood was collected at 2 h after LPS (2 mg/kg) injection and serum was subjected to TNF-α assay. Results are represented as the mean ± SD (n=4-10). **p<0.001, *p<0.05, Significantly different from LPS-treated group.

LPS-stimulated TNF-α production, showed about 50% reduction in serum TNF-α level at the concentration of 2 mg/kg.

Next we have investigated the effects of GTS dosing time (Fig. 2). GTS, pretreated 3 hr before LPS, also showed the inhibitory effect on LPS-induced TNF-α production. However, when GTS was administered at 30 min after LPS injection, the TNF-α level after 1.5 hr after GTS injection was much less reduced as compared with that of coadministered GTS. Therefore, it seems that GTS has no effect on the previously activated signaling path-

Table 1. The comparison of serum TNF-α level by p.o. and i.p. treatment of GTS

Treatment	Serum TNF-a (ng/ml)	n
Saline	0.839±0.780	7
LPS only[b]	6.980±2.990	16
LPS+GTS (i.p.)[c]	0.875±0.974	10
LPS+GTS (p.o.)[c]	4.200±1.161	10
LPS+Dexamathasone (i.p.)[b]	2.750±1.196	6

[a] Represents the mean±S.D.
[b] Doses and method of treatments were described in Materials and Methods section.
[c] GTS (50 mg/kg) was coadministered with LPS.

ways.

Since ginseng has been used as an oral remedy for many years, we tested whether orally administered GTS has the inhibitory effects on serum TNF-α elevation induced by LPS. The serum TNF-α was not significantly reduced by oral administration of GTS (Table 1). This might be due to the delayed absorption of GTS from gastrointestinal tract, since the serum TNF-α was rapidly induced by LPS (T_{max} was 2 hr in our experiment) and GTS could not inhibit the TNF-α production from already activated mice. Metabolic inactivation of the active component of GTS can be another possible reason for oral GTS having less activity. Recently, it has been noted that metabolic derivative of protopanaxadiol showed antimetastatic or antitumor activity.[21-22] Therefore, it is necessay to identify active compounds that show inhibitory effect on serum TNF-α production and to evaluate the effects of the metabolic products of GTS. Further investigation is also necessary to clarify the exact mechanism for the action of saponins on the production of TNF-α and their biological application. The serum TNF-α levels after various treatments (i.p. and p.o.) with GTS are summarized in Table 1.

In summary, serum TNF-α level of LPS-stimulated mouse was remarkably reduced by intraperitoneal administration of GTS. This study provides the clue for elucidating the protective effect of extract of ginseng root against TNF-α-mediated injury.

요 약

사포닌은 인삼의 약리작용을 매개하는 인삼의 주요 성분이다. 인삼의 뿌리는 여러 가지 독소에 의하여 유발되는 독성에 대한 보호작용이 있는 것으로 알려지고 있다. 본 연구에서는 세균독소(lipopolysaccharides)에 의하여 유도되는 종양괴사인자 생성에 미치는 인삼 사포닌의 영향을 살펴보았다. 독소를 투여한 생쥐의 혈청중 종양괴사인자의 농도는 상승하는데, 인삼 사포닌 50 mg/kg을 독소와 함께 복강으로 투여한 경우는 독소만 투여 하였을 때에 비하여 혈정 중 종양괴사인자의 농도가 현저히 낮았다. 이러한 인삼 사포닌의 효과는 사포닌의 용량, 투여방법과 투여시간에 영향을 받았는데, 사포닌을 경구로 투여하거나 독소를 주사한 후에 투여하였을 때에는 종양괴사인자 농도에 유의성 있는 차이를 나타내지 않았다. 이러한 결과는 인삼이 간 손상에 대한 보호작용이 있을 가능성을 시사하였고, 사포닌이 이러한 인삼의 보호효과를 매개하는 활성 성분임을 의미한다.

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