Ginseng Extract Protects Unsaturated Fatty acid from Decomposition Caused by Iron-Mediated Lipid Peroxidation

Shigeru Okada and Daxian Zhang

Department of Pathology, Okayama University Medical School 2-5-1 Shikata, Okayama 700-8558, Japan

ABSTRACT

We hypothesized the primary effect of ginseng was to protect cell membrane fatty acids from decomposition caused by free radicals. To confirm the antioxidant effect of ginseng, we measured the inhibitory effect on the formation of thiobarbituric acid-reactive substances, an indicator of lipid peroxidation, and evaluated the free radical scavenging effect of ginseng by electron spin resonance spectrometer, and gas chromatography. The results showed that thiobarbituric acid-reactive substances formed and the loss of arachidonic acid during lipid peroxidation, and that hydroxyl(-like) radical peak formed by the iron complex (ferric nitrilotriacetate, an known free radical generator *in vivo*) were completely inhibited by ginseng extract. This antioxidant effect of ginseng may be responsible for its wide pharmacological actions in clinical practice. As the free radical reactions in general are rapid and non-specific, ginseng seems to act as a normalizer, rather than a general tonic, at the stages of acute or chronic active phase of the various diseases.

Introduction

Panax ginseng is an herbal root that has been used in clinical practice for more than four thousand years in folk medicines. It is most often used as a general tonic. Recently, more of its effects have been attriduted to its antioxidant action.[1] Not only primary free radicals, e.g. hydroxyl (-like) radicals, peroxyl radicais, or alkoxyl radicals, but secondary products of hydroperoxyl fatty acids and their degradation products, e.g. various aldehydes are highly cytotoxic. They react with different kinds of biomolecules and finally degrade various tissues.[2] To confirm the antioxidant effect of ginseng, we measured thiobarbituric acid-reactive substances (TBARS), an indicator of lipid peroxdaition, evaluated the scavenging effect of ginseng by electron spin resonance spectrometer and gas chromatography in a free radical generating system using ferric nitrilotriacetate (Fe-NTA).[2]

Materials and Methods

Chemicals

All the reagents were of the highest quality available. The double distilled water further purified by using Milli-Q Labo (Millipore Ltd, Tokyo, Japan) was used throughout.

Ginseng extract preparation

The preparation of the ginseng aqueous extract was the same with that in clinical administration. Namely, commercially available ginseng was previously smashed, and the powder was soaked with water (1:25, w/w) in a glass flask for 3 hours, then the container was put on boiling water for 40 minutes. After filtration, the solid residue was subjected to the same process once again, then two filtrates were mixed and ice-dried.

The dried extract equaled about 20% (w/w) of the raw drug and 80 ng of iron contained per gram of ginseng extract (measured by an atomic absorption spectrophotometer, Hitachi Z-9000). Before use, the extract was diluted to the desired concentration.

Measurements of TBARS

Lipid peroxidation was carried out by incubation at 37℃ with shaking.[4] A 2 ml sample contained 0.1 M HEPES-NaCl buffer (pH 7.3), 10% methanol, 50 mM of arachidonic acid, 50 mM Fe-NTA, and 50 mM hydrogen peroxide. To observe the antioxidation effect of ginseng extract, ginseng extract was further added, and the antioxidation effect was compared with that of α-tocopherol, a known radical scavenger.[5] After the desired incubation time at 37℃ with shaking, TBARS production was measured by the method of Buege and Aust.[6]

Gas chromatography study

Lipid peroxidation was induced by the same method as that for measurements of TBARS. After the desired periods of incubation, the lipids in the mixture were extracted twice with cyclohexane and diethyl ether, followed by evaporation under a stream of nitrogen gas. Finally, the lipid was subjected to trimethylsilylation at room temperature. Gas chromatography for analysis of fatty acid was carried out with a Hitachi G-3000 chromatogragh equipped with a flame ionization detector, using an OV-1 fused-silica capillary column, The carrier gas was helium, and the peak area integration was achieved with a Hitachi 833 data processor. The quantification of unsaturated fatty acid loss were expressed as ratio of arachidonic acid (C=20:4) and nonadecanoate (C=19:0).

ESR study

Hydroxyl(-like) radicals were measured by the spin trapping method using 5,5-dimethy1-1-pyrroline N-oxide(DMPO). The aqueous mixtrue was added to a disposable glass micropipette for detection by an ESR spectrometer (JES-FE1XG, JOEL Co. Ltd., Tokyo). To observe the antioxidation effect of ginseng extract, the ginseng extract diluted with HEPES buffer was added to the free radical producing system. HEPES buffer, glucose solution diluted with HEPES buffer, or starch solution diluted with HEPES buffer were used in place of HEPES buffer in the mixture. The final concentration of ginseng extracts, glucose, and starch in the mixtures was 0.25%, 0.05%, 0.25%, and 0.25%,

respectively. The measurements were repeated at least three times, and the average was used for the data analysis.

Absorption spectrophotometer study

Our intention was to observe whether chelate structure of the iron complex was affected by ginseng extract, namely, whether the iron-sequestration mechanism was responsible for its inhibition of lipid peroxidation and peroxyl radical formation. Ferric nitrilotriacetate and 0.1M HEPES buffer (pH 7.0) with or without ginseng extract was measured by absorption spectrophotometer.

Results and Discussion

Measurements of TBARS

TBARS was elevated in unsaturated fatty acid samples undergoing lipid peroxidation in the absence of ginseng extract or α-tocopherol. In contrast, TBARS production was inhibited by the presence of α-tocopherol or a large dose (0.05%) of ginseng extract. Starch and glucose as controls did not inhibit the iron-induced increase in TBARS production (Fig. 1). The same dose of ginseng extract added at 3 hour of the incubation also inhibited TBARS production, but a small dose (0.01%) of ginseng extract did not inhibit TBARS production (data not shown).

The ability of ginseng to inhibit fatty acid from decomposition due to iron-mediated lipid peroxidation was shown by this study. The finding that ginseng inhibited TBARS production even after 3 hours of incubation suggests that the antioxidant effect of ginseng extract might act not only at the initiation of the radical chain reaction but also at the propagation stage. To eliminate possible radical-quenching action by nonspecific macromolecular substances, solutions of starch and glucose were used as controls, but no antioxidant effects were seen.

Gas chromatography study

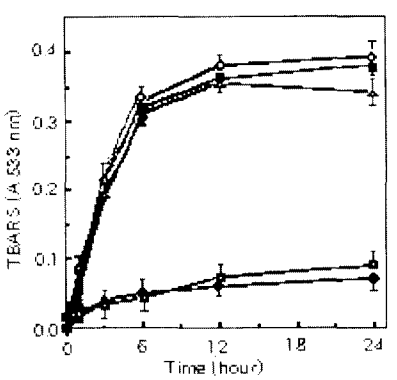

Fig. 1
TBARS in time course of lipid peroxidation generated by 50 μM arachidonic acid with 50 μM of Fe-NTA and 50 μM of H2O2. The treatments are: ○ no addition, ■ 0.05% glucose, △ 0.05% starch, □ 0.005% α-tocopherol, ● 0.05% ginseng extract

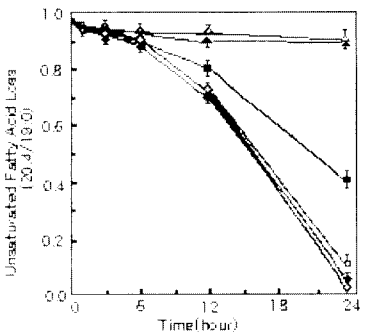

Fig. 2
The analysis of gas chromatography for gineseng extract protecting unsaturated fatty acid from decomposition. Lipid peroxidation was conducted as in Fig. 1. Quantifications of unsaturated fatty acid loss are expresed as ratio of archidonic acid (20:4) and nonadecanoate (19:0). The treatments are : 0.005% α-tocopherol, ▲ 0.05% ginseng extract, ■ 0.01% ginseng extract, □ 0.05% starch, ○ 0.05% glucose, ● no addition

The results of analysis of fatty acids by gas chromatography are shown in Fig. 2.

The samples undergoing lipid peroxidation in the absence of α-tocopherol or a large dose (0.05%) of ginseng extract showed the unsaturated fatty acid loss over time, but in the presence of them the unsaturated fatty acid loss was inhibited. Unsaturated fatty acid loss was not completely inhibited by the small dose (0.01%) of ginseng extract.

These study also support the the ability of ginseng to inhibit decomposition of fatty acid due to iron-mediated lipid peroxidation.

ESR study

The results showed that 0.25% ginseng extract completely counteracted the DMPO-OH generation in aqueous reaction system. Even at a dose of 0.05% ginseng extract, it still showed radical-scavenger effect, although the action becomes weak. But the starch and glucose as controls showed no radical-scavenger effect at the same dose with ginseng extract (Fig. 3)

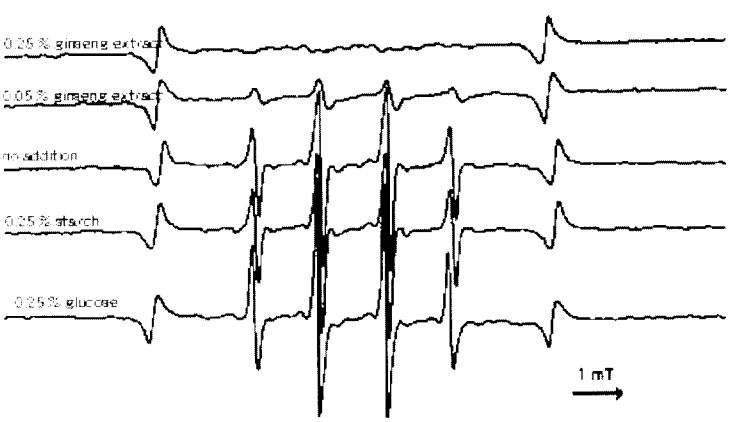

Fig. 3. The ESR study for hydroxyl radical-scavenger effect of ginseng extract. A reaction mixture containing final concentration of 0.58mL Fe-NTA, 2.5mM H_2O_2, 50mM DMPO, and 0.2 M HEPES buffer (pH 7.0)

In general, the iron-catalyzed lipid peroxidation is thought to be mediated by hydroxyl radicals generated from hydrogen peroxide *via* a Fenton reaction,[7] although there is debate on this point.[8] We also observed the hydroxyl radical-scavenging effect of ginseng in the ESR study under a ferrous ion-mediated Fenton reaction at acidic condition (pH 4.5), in which the ginseng extract is thought to hardly chelate with iron, and hydroxyl radicals are generated more easily than under neutral conditions. When 0.25% of ginseng extract was added to the mixture, the DMPO-OH signal was about 50% smaller(data not shown).

Absorption spectrophotometer study

The results of the absorption spectrophotometer studies show that the characteristic absorbance peaks of the Fe-NTA are eliminated by adding an ample dose (2.5%) of ginseng extract (dose ratio of iron and ginseng extract was equal to that of ginseng inhibition of hydroxyl radical in ESR study, Fig. 3). A smaller dose (0.10%)of ginseng extract did not change the pattern of characteristic peaks of Fe-NTA abviously (Fig. 4). Fig. 4

The results of the absorption spectrophotometer studies showed that the characteristic absorbance peaks of the ferric chelate are eliminated by adding ginseng extract; this suggests that the chelate structure of Fe-NTA was affected by ginseng extract, namely ginseng extract also may bind to ferric complex under neutral pH.

Thus, in this study, the mechanism of the antioxidation activity of ginseng extract may, in part, be due to directly scavenging hydroxyl (-like) radical and sequestering iron.

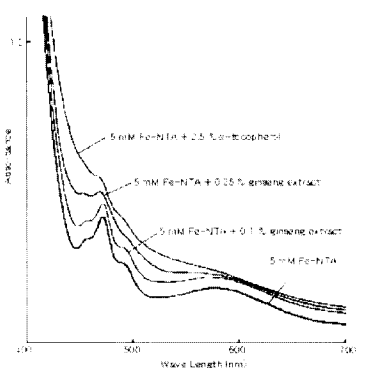

Fig. 4. The characteristic absorbance of the Fe-NTA affected by ginseng extreact with various concentrations. Iron was contained as indicated.

As the free radical reactions in general are rapid and non-specitic, ginseng seems to act as a normalizer, rather than a general tonic, at the stages of acute or chronic active phase of the various diseases.

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