THE EFFECT OF THE SAPONIN FRACTION OF *PANAX GINSENG* C.A. MEYER ON THE ANTIOXIDANT ACTIVITY OF TOCOPHEROL

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ABSTRACT

The effect of the saponin fraction extracted from Panax ginseng C.A. Meyer on the antioxidant activity of α-tocopherol was investigated *in vitro* as well as *in vivo*.

Microsomal preparation of albino rat (Sprague-Dawley, 180-200g) was incubated in the mixture containing NADPH, Fe^{3+}, ATP, α-tocopherol with and/or without ginseng saponin fraction for 30 minutes and the malondialdehyde formed was assayed and found that the saponin fraction stimulated the antioxidant activity of α-tocopherol cooperatively. It was also realized that the cooperative stimulation of the antioxidant activity of α-tocopherol was most eminent when the concentration of the saponin fraction was around 10^{-5}% in the reaction mixture.

Alcoholic suspension of α-tocopherol with and/or without ginseng saponin fraction was administered orally to rats in which the lipid peroxidation was induced by ethanol administration and the lipid peroxide contents of the liver were assayed at certain periods of time after α-tocopherol administration in this animal.

It was reported that the saponin fraction stimulated the absorption of α-tocopherol in rats and this was confirmed again in the present work.

From the previous work and present experimental results, it seemed that the saponin fraction accelerated the absorption of α-tocopherol and therefore stimulated the antioxidant activity of α-tocopherol more effectively in the animal body.

INTRODUCTION

It is well known that the function of vitamin E in metabolism, the primary, if not the sole, is that of *in vivo* lipid antioxidant. Probably the most direct evidence to substantiate this theory is that lipid peroxide have been found in the tissue of vitamin E-deficient animals. It is assumed that vitamin E acts as an *in vivo* lipid antioxidant, protecting unsaturated fatty acids in tissue lipids against peroxidation.[1]

Of the naturally occuring tocopherols, α-tocopherol is the most common and in terms of biological activity, is also the most potent in relieving vitamin E deficiency symptoms.[2,3]

Tocopherol has been shown to be absorbed via the lymphatic pathway and transported in lymph and plasma bound to lipoproteins and taken up from plasma by tissues such as liver and kidney.[4]

Recently it was demonstrated that absorption of α-tocopherol in rat was prompted by ginseng saponin fraction when α-tocopherol fed with ginseng saponins orally.[5,6]

This paper described that effect of the saponin fraction of panax ginseng C.A. Meyer on the absorption of α-tocopherol and its antioxidant activity *in vitro* as well as *in vivo*.

MATERIALS AND METHODS

Total 17g of ginseng saponin mixture was obtained from 300g of powdered Korean white ginseng (Keumsan, 4 years, 50 pieces) according to the modified procedure described elsewhere.[7] The chromatogram of the saponin showed that it contained several saponins with R_f values of 0.71, 0.65, 0.59, 0.52, 0.47, 0.43, 0.41, 0.36, 0.34, 0.27, 0.22, 0.20, 0.17 on silica gel pre-made thin plate (pre-coated TLC plates, silica gel 60 F-254) by using chloroform-methanol-H_2O (65:40:9, v/v/v) as a developing solvent. It appeared that the saponins with R_f values of 0.22, 0.27 were the most abundant, the saponins with R_f values of 0.47, 0.43, 0.36, 0.34, 0.20, 0.17 were less abundant and the saponins with R_f values of 0.71, 0.65, 0.59, 0.52 were the least. The above saponin mixture was used without further purification for this study.

The livers of albino rats (Sprague-Dawley, 160-200g) were homogenated in 0.15M Tris-HCl buffer (pH 7.4) and made up to 20% homogenate and a microsomal fraction was obtained according to Fairhurst et al.[8]

An *in vitro* antioxidant activity was measured as follows. Reaction mixture (2.5ml) contained (final concentration) 0.1mM $FeCl_3$, 1.7mM ADP, 0.4mM NADPH, various concentrations of α-tocopherol (0 – 100μM) and ginseng saponin fraction (0 – 10^{-2}%) and microsome preparation. After 30 minutes incubation, the reaction was terminated by adding 2ml of 30% trichloroacetic acid and then 2ml of 0.75% thiobarbiturate (TBA) and 0.2ml of 5M HCl were added, mixed, heated in boiling water for 15min. and then cooled. After centrifugation, the optical density at 535nm of the supernatant was read and the malondialdehyde (MDA) formed was calculated according to Fairhurst et al.[8]

Proteins were determined according to Lowry method.[9] For an *in vivo* experiments, the rats were dosed 12% ethanol (free access) instead of water with/without α-tocopherol (500μg) and/or ginseng saponin fraction (1mg) for 6 days. On the seventh day after 24 hours fasting, the livers were taken up and 10% homogenates were prepared and the amounts of lipid peroxides were determined according to Ohkawa.[10] To 0.2ml of 10% liver homogenate, 0.2ml of 8.1% SDS,

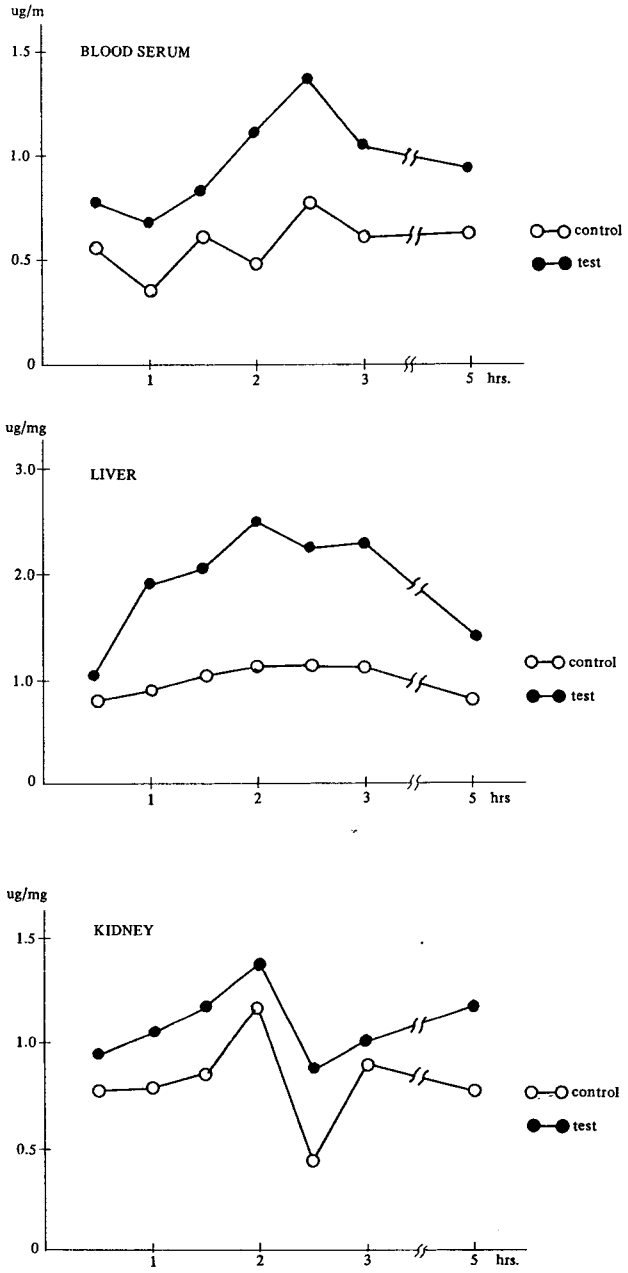

Fig. 1. The effect of ginseng saponin fraction on the absorption of α-tocopherol in serum, liver and kidney of rats fed orally with α-tocopherol (500 μg) containing D-α-5-methyl-^{3}H-tocopherol (502,000 cpm) with/without 1mg of ginseng saponin fraction on time course. The values (average of 5 rats) are expressed as μg/ml (serum) or μg/mg (liver and kidney) calculated from the ^{3}H activity.

Table 1. Effect of α-tocopherol on malondialdehyde formation.

Treatment	Rate of lipid peroxidation (nmoles MDA/mg protein/min)	%
None (control)	1.31 ± 0.165	100
50 μM α-tocopherol	1.10 ± 0.085	84
100 μM α-tocopherol	0.71 ± 0.044	54

a: Values given are means ± SEM for five separate experiments.

Table 2. The effect of ginseng saponin fraction on microsomal lipid peroxidation.

Added saponin concentration(%)	Rate of lipid peroxidation* (nmoles MDA/mg protein/min)	%
0	1.29 ± 0.28	100
10^{-7}	1.26 ± 0.33	98
10^{-6}	1.25 ± 0.26	97
10^{-5}	1.14 ± 0.23	85
10^{-4}	1.17 ± 0.30	91
10^{-3}	1.34 ± 0.56	105

* The values are mean ± S.D. for seven separate separate experiments

Table 3. Effect of ginseng saponin fraction on antioxidant activity of α-tocopherol.

Treatment	Rate of lipid peroxidation n moles MDA/mg protein/min*	%
50 μM α-tocopherol (control)	1.17 ± 0.09	100
50 μM α-tocopherol + 10^{-2}% saponin	1.23 ± 0.09	105
50 μM α-tocopherol + 10^{-3}% saponin	1.10 ± 0.08	94
50 μM α-tocopherol + 10^{-4}% saponin	0.73 ± 0.05	62
50 μM α-tocopherol + 10^{-5}% saponin	0.43 ± 0.06	37
50 μM α-tocopherol + 10^{-6}% saponin	0.40 ± 0.08	34

a: Values given are means ±SEM for five separate experiments.

1.5ml of 20% acetate buffer (pH 3.5), 1.5ml of 0.8% TBA and distilled water were added to make up to 4ml of raction mixture. This was then heated at 95°C (water bath) for an hour, cooled, added 1ml of distilled water and 5.0ml of n-butanol-pyridine mixture (15:1, v/v) and then centrifuged. The optical density of the butanol-pyridine layer at 532nm was measured and the amounts of lipid peroxides was estimated with the reference of tetramethoxypropane.

DL-α-tocopherol was purchased from E. Merck Co. and D-α-5-methyl-^{3}H tocopherol was obtained from Amersham. Tris (hydroxymethyl) aminoethane (Tris), 2-thiobarbituric acid (TBA),

Table 4. The effect of ginseng saponin fraction on the antioxidant activity of α-tocopherol in the liver of rats dosed 12% ethanol (fre access) instead of water for 6 days. The values are mean values of five rats.

Group	Lipid peroxides (nmole/g liver)	Degree bf peroxidation (Relative %)	antioxidant* activity of the liver
Control 12% ethanol for 6 days	577	100	0.50
Test 1 0.1% saponin 12% ethanol for 6 days	474	82	0.61
Test 2 5×10^{-2}% α-tocopherol in 12% ethanol	337	58	0.86
Test 3 0.1% saponin + 5×10^{-2}% -tocopherol in 12% ethanol	239	42	1.19
Normal	289	50	1.00

* The antioxidant activity was expressed assuming that the activity of nomal group was 1.00

tetramethoxypropane(TMP), β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), adenosine-5'-diphosphate (ADP) were the products of Sigma chemical Co.

RESULTS AND DISCUSSION

It was demonstrated that the absorption of α-tocopherol was greatly prompted by ginseng saponin fraction when α-tocopherol (500ug) was fed with the saponin fraction (1mg) extracted from *panax ginseng* C.A. Meyer. This was confirmed again in the present study as shown in Figure 1. Maximum absorption peak appeared at 2-3 hours after the feeding in both control and ginseng saponin fed group and the amounts of α-tocopherol absorbed in blood, liver and kidney were always considerably more in ginseng saponin fed group than control rats.

Saponins are surface active and it is easily expected that the ginseng saponin fraction solubilize the fat soluble vitamin resulting in a rapid absorption of α-tocopherol.

As shown table 1, α-tocopherol prevented malondialdehyde formation when the microsome preparation was incubated in the reaction mixture containing Fe^{3+}, NADPH and ADP demonstrating the antioxidant activity of α-tocopherol *in vitro*.

Ginseng saponin fraction was also prevented malondialdehyde formation when the concentratioin of the saponin fraction was 10^{-5}% in the above reaction mixture but its antioxidant activity was much less than that of α-tocopherol as shown in table 2.

However, the saponin fraction stimulated the antioxidant activity of α-tocopherol cooperatively, particularly its effect was mos. eminent when the concentration of the saponin fraction was 10^{-6}-10^{-5} % in the reaction mixture as shown in table 3. At the higher concentration of the saponin fraction, the formation of malondialdehyde seemed to be increased suggesting that the exess amounts of saponins might be unfavour for the antioxidant activity of α-tocopherol.

It was demonstrated that when 1mg of

ginseng saponin was given orally to a rat, about 17% of the saponin was found absorbed and the concentration of the saponin in blood and liver would be approximately 10^{-5}% level at 2 hours after the feeding and gradually disappeared.[7]. Under this conditions, most enzymes were known to be stimulated and the antioxidant activity of α-tocopherol would be most active.

In vivo experiment showed that the amounts of lipid peroxide of the liver of α-tocopherol (500ug plus ginseng saponin (1mg) group was much less than α-tocopherol (500ug) but no ginseng saponin group suggesting again that the saponin fraction stimulated the antioxidant activity of α-tocopherol considerably as shown in in table 4.

From the above experimental results, it was concluded that the saponin fraction of panax ginseng C.A. Meyer has a little antioxidant activity itself but stimulate cooperatively the antioxidant activity of α-tocopherol significantly. Furthermore, the saponin fraction stimulate the absorption of α-tocopherol resulting in a double effect of antioxidant activity of α-tocopherol.

Cho: In your experiment you measured malondialdehyde *in-vitro* and peroxide *in-vivo* in order to see if there is any possible cooperative effect of ginseng. Is there any specific reason why you used two different methods?

Hong: Measuring method for malondialdehyde formation has many interference *in-vitro* but this experiment shows only relative comparison between control group and saponin and tocopherol fed groups. So I think that the interference on this result is almost same. But *in-vivo*, we used Yagi method and it has reported that according to Yagi method the interference caused by many reasons is minimized.

한국산 인삼(Panax ginseng C. A, Meyer)의 사포닌 성분이 α-tocopherol의 항산화작용에 미치는 영향

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한국산 인삼(Panax ginseng C. A. Meyer) 뿌리에서 추출한 사포닌 성분이 α-tocopherol의 항산화 활성에 미치는 영향을 실험관 내 실험과 동물실험으로 관찰하였다.

흰쥐(Sprague-Dawley, ♂, 180~200g)의 마이크로좀 분획을 NADPH, Fe^{+3}, ATP, α-tocopherol 과 인삼 사포닌 분획(대조군은 사포닌 분획대신 같은 부피의 물)을 함유한 혼합물에서 30분간 반응을 진행시키고 생성되는 molondialdehyde를 분석한 결과 사포닌 분획은 α-tocopherol의 항산화작용을 상승적으로 증가시켰다. 그리고 α-tocopherol의 항 산화작용에 상승적 효과는 반응액에서의 인삼 사포닌 분획의 농도가 10^{-5}% 전후에서 특히 현저하였다.

술로 과산화물을 유발케 한 쥐에게 α-tocopherol만을 또는 α-tocopherol과 인삼 사포닌 분획의 혼합물을 경구투여하고 일정 시간 후에 간의 과산화 지질을 thiobarbituric acid 방법으로 분석한 결과 사포닌 분획이 동물체내에서도 α-tocopherol의 항 산화작용을 크게 촉진시키는 것으로 관찰되었다. 또한 사포닌 분획은 α-tocopherol의 흡수를 촉진시키는 것으로 보고되고 있으며 본 실험에서도 이것을 확인하였고 이와 같은 사포닌 분획의 α-tocopherol 흡수 촉진은 α-tocopherol의 항 산화작용을 더욱 효과적으로 촉진시킬 것으로 생각된다.

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