EFFECTS OF GINSENOSIDE Rb_2 ON THE ANTIOXIDANTS IN SENESCENCE-ACCELERATED MICE(SAM-R/1)

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ABSTRACT

In biological system, there are enzymes such as superoxide dismutase(SOD), catalase and glutathione(GSH) peroxidase which scavenge reactive oxygen species as well as antioxidants such as ceruloplasmin, albumin and nonprotein-bound SH including GSH related to defense mechanism. In the present study, the protective effects of ginsenoside Rb_2 against oxidative stress were investigated in the SAM-R/1 mice. Treatment with ginsenoside Rb_2 significantly increased Cu, Zn-SOD and Mn-SOD in the liver. Ginsenoside Rb_2 tended to increase hepatic catalase activity and significantly increased serum albumin and nonprotein-bound SH levels in the liver. But treatment with ginsenoside Rb_2 showed a significant decrease in hepatic malondialdehyde(MDA) levels compared to control group. Furthermore, we compared the effects in the hepatic SOD, MDA and serum albumin. These findings suggest that the increase of antioxidants by ginsenoside Rb_2 results in the protective effects against reactive oxygen species.

INTRODUCTION

Harman(1) proposed that damage arising from free radical reaction was the mechanism responsible for aging. Oxygen-derived species such as $\cdot O_2^-$, $HO\cdot$, H_2O_2 and other products of free radical and lipid peroxidation reactions have been implicated in the mechanism of several age-associated disorders, among them atherosclerosis, cancer, rheumatoid arthritis, emphysema, amyloidosis, senile cataract and Parkinson's disease as well as in the decline of immune function. Indeed, oxygen free radicals are highly reactive molecules with unpaired electrons, which are produced within aerobic cells in the course of normal metabolic events.

Normally, the aerobic cells are protected from the damage of free radicals by various antioxidant enzymatic system such as superoxide dismutase, catalase and glutathione peroxidase. For any reason, a diminished activity of these enzymes can cause an increase of lipid peroxidation, protein oxidation and DNA damage.

In these research, we investigated the age-associated alteration in antioxidant enzyme activities and effects of ginsenoside Rb_2 on the antioxidants in senescence-accelerated mice(SAM-R/1).

MATERIALS AND METHODS

Materials : All chemicals were purchased from Sigma Chemical Company. Ginsenoside Rb_2 was isolated and purified from a root extract of *Panax ginseng* C.A., Meyer produced in Kumsan, Korea.

Animals : Senescence-accelerated mice(SAM-R/1), which were developed by Dr. Takeda in kyoto University, were used for these experiments.

Treament : Ginsenoside Rb_2 intraperitoneally treated in a dose of 2.5, 5.0 mg/kg/day or 10μmol/kg/day for 5days.

Malondialdehyde(MDA) : MDA was assayed by measuring thiobarbituric acid(TBA) reactive material(2).

Superoxide dismutases(SOD) : SOD served as a first line of defense by dismutating superoxide anions. The cytosolic Cu-Zn SOD was assayed in the absence of cyanide. The Mn-SOD was assayed in mitochondrial fraction in the presence of cyanide to inhibit the cytosolic SOD. The detection of $\cdot O_2^-$ by oxidation of hydroxylamine yielding nitrite was measured by colorimetric reaction(3).

Catalase : The function of catalase was to catalyze the decomposition of the H_2O_2 and organic peroxides formed by free radical reactions. The activity of catalase was assayed in both homogenates and cytosols as described(4) using H_2O_2 as a substrate.

GSH : The GSH concentrations in the acid soluble fractions was determined by the GSH reductase/DTNB recycling method of Tietze(5).

Nonprotein-bound SH : Aliquots of 5.0 ml of the homogenates was mixed in 15.0 ml test tubes with 4.0 ml distilled H_2O and 1.0 ml of 50% trichloroacetic acid (TCA). Two ml of supernatant was mixed with 4.0 M tris buffer (pH 8.9), 0.1 ml DTNB added, and the sample shaken. The absorbance was read within 5 min of the addition of DTNB at 412 mμ against a reagent blank with no homogenate(6).

Protein-bound SH : The protein-bound SH was calculated by substracting the nonprotein-bound SH from total bound SH (6).

RESULTS AND DISCUSSION

Age-associated alteration in the hepatic SOD and catalase : Cu/Zn-SOD activities decreased during aging in SAM-R/1(Fig. 1), whereas there were no change in Mn-SOD activities with age. Furthermore, catalase activities decreased with age until 5 months of age and then there were no changes.

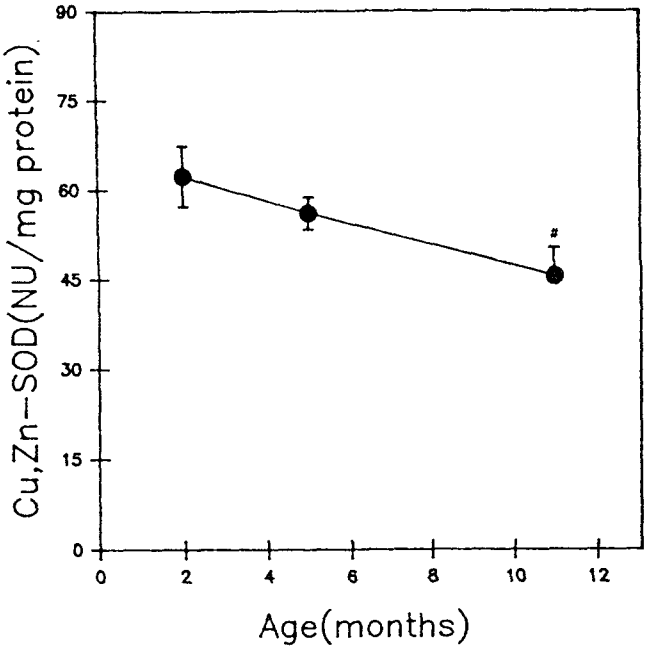

Fig.1. Cu, Zn - Superoxide dismutase(SOD) activities in SAM - R/1 at 2, 5 and 11 months of age. Data are means± S.E. from 5 animals. Statistical significance : p<0.01 vs. 2 months of SAM - R/1.

Antioxidant effects of ginsenoside Rb_2 : As shown in Table I, treatment with ginsenoside Rb_2 significantly increased the hepatic Cu, Zn - SOD and Mn - SOD. Ginsenoside Rb_2 caused a significant increase in nonprotein - bound SH, whereas there were no changes in protein - bound SH levels(Table II). As shown in Fig. 2, administration of ginsenoside Rb_2 significantly decreased in hepatic MDA levels compared to control group.

Table I. Effect of ginsenoside Rb_2 on SOD activity in the liver of SAM - R/1 mice.

Group	Mn - SOD (NU/mg protein)	Cu, Zn - SOD (NU/mg protein)
Control	4.25± 0.08	25.62± 2.19
Ginsenoside Rb_2 (2.5 mg/kg)	5.05± 0.19**	34.42± 2.08*
Ginsenoside Rb_2 (0.5 mg/kg)	4.86± 0.16**	34.04± 2.17*

Values are means± S.E. of 6 mice.
Statistical significance : * P<0.05, ** P<0.01 vs. control group.
Ginsenoside Rb_2 was intraperitoneally treated in a dose of 2.5 or 5.0 mg /kg/day for 5 days.

Table II. Nonprotein - bound and protein - bound sulfhydryl concentration in the liver of SAM - R/1 mice treated with ginsenoside Rb_2.

Group	Nonprotein - bound SH (μMol/g tissue)	Protien - bound SH (μMol/g tissue)
Control	6.54± 0.48	19.09± 0.63
Ginsenoside Rb_2 (2.5 mg/kg)	8.19± 0.54*	19.07± 0.34
Ginsenoside Rb_2 (0.5 mg/kg)	7.50± 0.25	18.76± 0.23

Values are means± S.E. of 6 mice.
Statistical significance : * P<0.05 vs.control group.

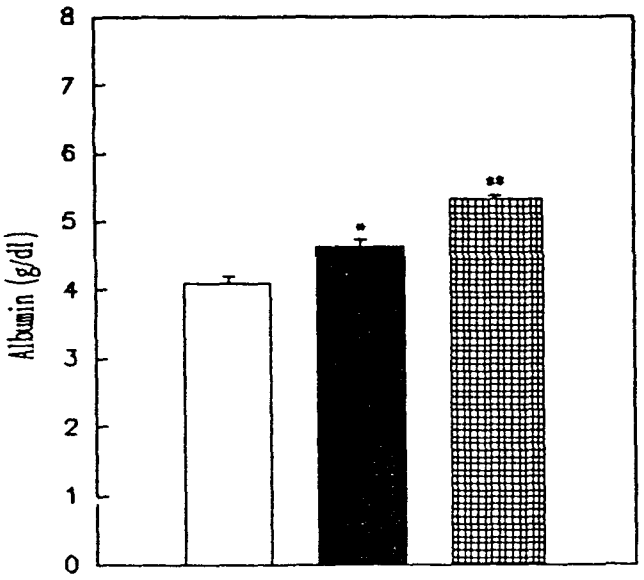

Fig. 2. Effects of ginsenoside Rb_2 or dexamethasone on the serum albumin in SAM - R/1 mice.
Control (□), Ginsenoside Rb_2 10 μM/Kg (■), Dexamethasone 10 μM/Kg (▦) ; Values are means± S.E. of 6 mice. Statistical significance : *P<0.05, ** P<0.01 vs. control group.

Steroid - like effects of ginsenoside Rb_2 : Hitting on the idea that ginsenoside Rb_2 has a similarity to glucocorticoid derivative in structure, we compared the effects of dexamethasone with that of ginsenoside Rb_2. As shown in Table III and Fig. 2, dexamethasone or ginsenoside Rb_2 increased Cu, Zn - SOD and serum albumin. In addition, treatment with ginsenoside Rb_2 or dexamethasone significantly decreased the hepatic MDA levels.

Table III. Effect of ginsenoside Rb_2 or dexamethasone on SOD activity in the liver of SAM - R/1 mice.

Group	Mn - SOD (NU/mg protein)	Cu, Zn - SOD (NU/mg protein)
Control	7.73± 0.07	24.26± 0.69
Ginsenoside Rb_2	8.04± 0.12	29.44± 1.85*
Dexamethasone	8.10± 0.35	28.48± 1.07*

Values are means± S.E. of 5 mice.
Statistical significance : * P<0.05 vs.control group.
Ginsenoside Rb_2 or dexamethasome was intraperitoneally treated in a dose of 10 μM/Kg.

Serum albumin is a major component of serum proteins, and it is known that albumin contributes to maintaining the osmotic pressure and is known also to carry a surplus of steroid hormones, thyroid hormones and metal ions. Recently, albumin was reported to have biological function as an antioxidant(7). Furthermore, serum albumin level fell progressively with each decade of age(8). However, treament with ginsenoside Rb_2 increased serum albumin as well as SOD and reduced MDA levels. These results suggest the possibility that ginsenoside Rb_2 might have the protective effect against active oxygen species, when their induction might be mediated through the steroid - like action.

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