Blood Superoxide Dismutase (SOD) Decrease Following Oral Administration of Plant SOD to Healthy Subjects

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We examined what changes occurred in the activity and content of superoxide dismutase (SOD) in the blood and the amount of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the urine as a consequence of oral administration of antioxidant health foods including plant-based SOD, vitamin C and vitamin E to seven healthy subjects every day for 15 days. Although there was a significant increase in the concentration of vitamins C and E in serum, there was a significant decrease in SOD (extracellular type) activity and Mn-SOD (mitochondrial type) content and a narrower range of variation therein. In contrast, there was a tendency toward an increase in the amount of 8-OHdG in the urine (observed in 6 of 7 subjects). We looked into the possibility that SOD activity was being inhibited by pycnogenol (water extract of the bark of the French maritime pine) as the main ingredient of the antioxidant health foods, and it became clear that SOD activity is included in pycnogenol. These results suggest that oral administration of antioxidant health foods containing SOD originating in plants has the effect of lowering the activity and content of SOD in the blood.

Key words —— antioxidant health food, plant-based SOD, urinary 8-OHdG in the urine, antioxidant vitamin, smoker

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INTRODUCTION

Since smokers are constantly exposed to excessive oxidative stress, it has been pointed out that the state of the oxidant-antioxidant balance in the body tends toward a predominantly oxidant state.[1,2] Thus, blood concentrations of vitamin C and other antioxidants (vitamins and minerals) decrease due to accelerated consumption in the blood and the amount of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the urine increase.[1–3] Oxidative stress induces cell injury including DNA oxidation, peroxidation reactions in lipids, inactivation and denaturation of proteins, *etc.* and proinflammatory reactions, and these are important factors that contribute to lifestyle-related diseases among smokers.[4,5]

We therefore carried out studies on healthy subjects (students including smokers and non-smokers) in order to detect what changes occurred in the state of oxidative stress (the oxidant-antioxidant balance) as a consequence of oral ingestion of antioxidant health foods including superoxide dismutase (SOD) of plant origin and vitamins C and E.

MATERIALS AND METHODS

Subjects —— The subjects were seven male students aged between 20 and 28 from this university comprising four smokers (smoking between 5 and 20 cigarettes a day with a smoking history of between two and nine years) and three non-smokers. Two of the subjects were in the habit of consuming health foods every day. They were requested to cease consuming these foods before the start of the test and to continue until the end of the test. The test subjects were subjected to informed consent. This research was examined and approved by the Ethics Committee of the Kawasaki University of Medical Welfare (No. 018).

Ingestion of Health Foods —— The three health foods used on this study were: Acerose C500, a food product containing vitamin C [500 mg of vitamin C/packet (also including sweetener, corn starch: 2 g/packet)]; Life Oil E and F, a food product containing vitamin E [vitamin E 200 mg/granule (also including safflower oil, soybean oil, brown rice germ oil, and glycerin, *etc.*: 880 mg/granule)]; Bell Pine Pure, a food product made from processed pine bark [pycnogenol,[6–8] 30 mg/granule and gliadin-combined plant SOD extract: oxykine,[9,10] 150 mg/granule (also including

lactose, yeast, vitamins C, E and B2, and wheat, *etc.*: 300 mg/granule)], all products being supplied by Kyowa Hakko Chemicals Co., Ltd., Toyama, Japan. Every day over a period of 15 days, in the morning and the evening, the subjects took one packet of Aserose C500 and one granule of Life Oil E and F as well as one granule in the morning and two granules in the evening of Bell Pine Pure. Accordingly, the quantities of the main ingredients ingested every day were 1000 mg of vitamin C, 400 mg of vitamin E, 90 mg of pycnogenol, and 450 mg of oxykine. A questionnaire survey on the state of the subjects' health was carried out before ingestion of the health foods and during the first and second weeks after the start of ingestion, and instructions were issued that the state of ingestion of these foods and any changes in the state of health should be recorded.

Blood samples and urine samples were taken on an empty stomach on the morning of the day on which ingestion of the health foods was due to begin and on an empty stomach on the morning of the sixteenth day (final ingestion incurred on the evening of the previous day).

Blood and Urine Tests —— 15 ml of elbow venous blood was sampled on an empty stomach on the morning before start of ingestion of the health foods, and measurements were taken of the concentrations of vitamin C, vitamin E and β-carotene in the serum employing the HPLC method,[11] the fluorescence method,[12] and the HPLC method,[13] respectively. SOD (extracellular type) activity in the plasma was measured using the improved nitrous acid method,[14] and the immunological content of Mn-SOD in the serum (mitochondrial type: enzyme protein content) was measured using the ELISA method.[15] Standard values for these tests were assumed on the basis of each of these methods already reported.[11–15] As an indicator for the state of oxidation, the amount of 8-OHdG in the urine (DNA oxidation product: ng/ml, corrected using creatinine in the urine) was measured using the ELISA method (standard values based on 2 to 30 ng/ml).[16]

Statistical Processing —— The results obtained were expressed in the form of average values ± S.D., and measurement of significant difference was carried out using the student t test, with $p < 0.05$ being considered to be a significant difference.

RESULTS

The present study came up with no significant differences between smokers and non-smokers in connection with items such as SOD activity in plasma, the content of Mn-SOD in the serum, the concentrations of vitamin C and vitamin E in serum, and the amount of 8-OHdG in the urine (Fig. 1). Furthermore, in comparison with the standard values described in the section of Materials and Methods, there were no items which smokers showed particularly low values, but SOD activity and the content of Mn-SOD were slightly higher than the standard values in the cases of both smokers and non-smokers (Fig. 1). Moreover, there was a positive correlation between SOD activity in plasma and the content of Mn-SOD in serum ($r = 0.91$, $p < 0.01$) and that there was a negative correlation between SOD activity in plasma (or the content of Mn-SOD in serum) and the amount of 8-OHdG excreted in urine ($r = 0.77$, $p < 0.05$; Fig. 2). This indicates that SOD has an important role to play in connection with the state of the oxidation-antioxidation balance in the body.

Significant changes before and after continuous ingestion of antioxidant health foods for 15 days were evident in the increase in serum vitamin C and vitamin E concentrations (both $p < 0.03$; Fig. 3), indicating that the test subjects had indeed been properly consuming the health foods. However, plasma SOD activity and serum Mn-SOD content, which had shown higher values prior to ingestion, had fallen in all cases to the standard values after ingestion ($p < 0.02$; Fig. 4). Moreover, whereas the range of variation in SOD activity before ingestion was between 2.8 and 8.5 (Δ5.7) U/ml, it became restricted to the narrower range of between 2.1 and 2.9 (Δ0.8) U/ml after ingestion. Similarly, in the case of Mn-SOD content, the range of 180 to 420 (Δ240) ng/ml prior to ingestion decreased to between 120 and 180 (Δ60) ng/ml after ingestion. This prompted us to study *in vitro* the influence of pycnogenol as the main ingredient of the antioxidant health foods on the activity of SOD in plasma, as a result of which we found that there was no evidence of pycnogenol inhibiting SOD activity and that there was a high degree of SOD activity in pycnogenol (Table 1).

The amount of 8-OHdG in the urine increased in six out of seven cases following ingestion of health foods, although there was no significant increase in the case of the mean values (20.6 ± 13.0 → 31.6

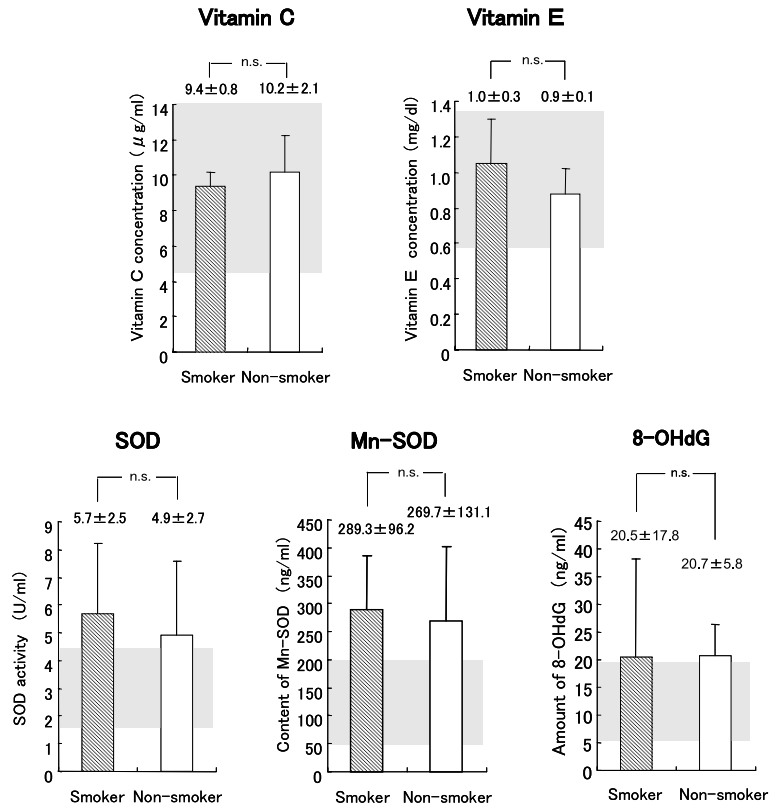

Fig. 1. No Differences between Smokers and Non-smokers in Serum Vitamin C and Vitamin E Concentrations, Plasma SOD Activity and Serum Mn-SOD Content and Urinary 8-OHdG Content

The shaded sections indicate standard values of the respective tests. Smokers (n = 4) and non-smokers (n = 3) before ingestion of antioxidant health foods. All are not significant (n.s.; p values from the left to the right are 0.59, 0.31, 0.72, 0.99 and 0.96, respectively).

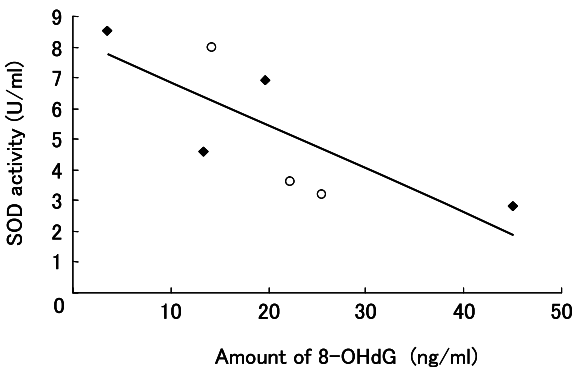

Fig. 2. Negative Correlation between Plasma SOD Activity and Amount of 8-OHdG in the Urine

◆, Smokers (n = 4); ○, Non-smokers (n = 3) before ingestion of antioxidant health food. $y = 0.141x + 8.272$ ($r = 0.77$, $p < 0.05$). Mn-SOD versus 8-OHdG (not shown in this figure): $r = 0.81$, $p < 0.05$.

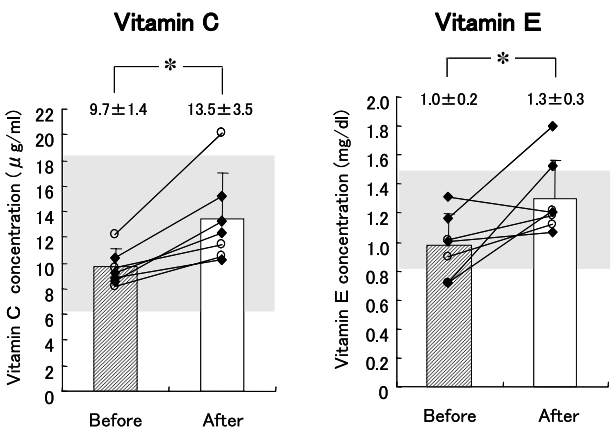

Fig. 3. Significant Changes of Serum Vitamin C and Vitamin E Concentrations before and after Ingestion of Antioxidant Health Foods

The symbols are the same as in Fig. 2. The shaded sections indicate standard values of the respective tests. Before versus after, $^{*}p < 0.05$.

± 19.4 ng/ml, p = 0.24; Fig. 4). No significant correlation was observed between the amount of variation in SOD activity before and after ingestion of health foods (the values prior to ingestion − the values after ingestion = ΔSOD) and the amount of variation of 8-OHdG (prior to ingestion − after ingestion = Δ8-OHdG) (r = 0.13, p = 0.78; Fig. 5). This indicates that the decrease in SOD activity after ingestion could not be said to have directly increased the amount of 8-OHdG.

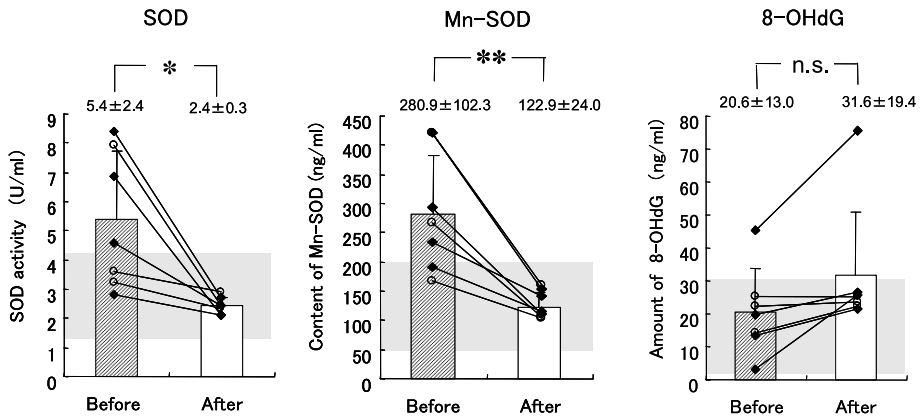

Fig. 4. Significant Changes in Plasma SOD Activity, Serum Mn-SOD Content and the Amount of 8-OHdG in the Urine before and after Ingestion of Antioxidant Health Foods

The symbols are the same as in Fig. 2. The shaded sections indicate standard values of the respective tests. Before versus after, $^{*}p < 0.02$, $^{**}p < 0.01$. 8-OHdG: n.s. ($p = 0.24$).

Table 1. SOD Activity in Pycnogenol Solution

Concentration of pycnogenol (mg/ml)	Added to plasma	SOD activity (U/ml)
0	−	less than 0.2
0.1	−	1.2
1.0	−	4.2
10.0	−	23.6
0	+	2.9
0.1	+	4.0

Pycnogenol solution (1, 10, 100 mg/ml) was added to plasma or water in a ratio of one to nine (final concentration: 0.1, 1.0, 10 mg/ml), and the SOD activity was measured according to the method described in the text. However, oxykine has poor solubility due to the presence of gliadin coating, and it was not possible to accurately measure SOD activity.

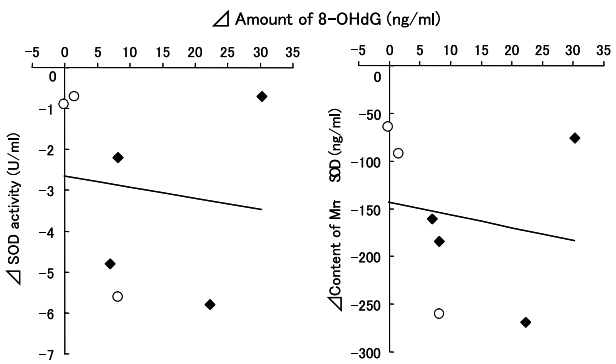

Fig. 5. No Correlation between the Amount of Variation in Plasma SOD Activity and Serum Mn-SOD Content and the Amount of Variation of 8-OHdG in the Urine before and after Ingestion of Antioxidant Health Foods

The symbols are the same as in Fig. 2. SOD activity versus 8-OHdG: $r = 0.13$, $p = 0.78$, and Mn-SOD content versus 8-OHdG: $r = 0.17$, $p = 0.71$.

DISCUSSION

Smokers are constantly exposed to oxidative stress and, in comparison with non-smokers, there is a fall in the concentration of antioxidant vitamins in serum due to the increased consumption of antioxidant substances.[17] As a result, it would seem that there is an increase in the concentration of thiobarbituric acid-reactive substances (TBARS), which are an indicator of the state of oxidation of an organism (an indicator of peroxides produced), of 8-OHdG in the urine and of 8-isoprostane (8-isoprostaglandin $F2_{\alpha}$, an indicator of peroxides on the cell membranes).[1] However, there were no items of antioxidant substances diminished significantly in the case of smokers, although there were few cases and a limited number of test items were included in the present study. This is probably due to the fact that the subjects in this study were not yet hardened smokers.

In the present study, contrary to expectations, oral ingestion of plant-based SOD brought about a decrease in plasma SOD activity (of the extracellular type). This type of SOD plays a role in protecting not only blood vessel tissue (its strong expression) but also other organs from oxidative stress.[18] Serum Mn-SOD (of the mitochondrial type) involves induction(enzyme protein *de novo* synthesized) responding to oxidative stress. Oral ingestion of melon (*Cucumis melo. L. C*) SOD extract (wheat gliadin-coarted: oxykine) reported that there is no break-up in the stomach or the upper digestive tract.[10] It penetrates the body through the M cells of the Peyer's patch, and thereafter SOD is exposed

from the coating of the wheat gliadin and is phagocyted by macrophage and dendritic cell, resulting the production of NO.[9,19] It has become clear that the production of SOD proteins inside the body is stimulated through Th1 cellular immunity (IFN_{γ}) and humoral immunity.[9,19] The results obtained on mice hitherto indicate that, with doses of 1 mg and 5 mg/mouse, activity of erythrocyte SOD (Cu/Zn-SOD) begins to increase from around a week after administration and reaches a plateau after the second week (approximately four times the previous values), and that hepatic SOD (Cu/Zn-SOD) activity also increases by around 3.5 times the previous values from the third week onward.[9,10] According to another report on mice,[20] causing exercise while breeding with oxykine additives brings about a much more conspicuous increase in hepatic SOD activity than in the case of breeding with normal food.

However, the results of oral ingestion over a three to four week period by human beings of oxykine (170 mg to 1500 mg/day) have shown that in every case that there is a decrease in erythrocyte SOD activity and that the conspicuous increase after exercise falls off.[21,22] These two studies have not been reported as an original article. More precisely, in the former case,[21] the study was concerned with Belgian professional footballers and it was found that activity prior to ingestion fell significantly with every subject from 1755 ± 367 IU/g hemoglobin (Hb) to within the standard value of 1138 ± 217 IU/gHb three weeks after ingestion of oxykine. In the latter case,[22] there was an increase from 1378 IU/gHb to 1477 IU/gHb before and after exercise prior to oral administration of oxykine, but following oral ingestion of oxykine there was no significant increase from the figure of 1289 IU/gHb recorded prior to exercise and that of 1302 IU/gHb recorded after exercise.

On the present investigation, after ingesting antioxidant health foods containing plant-based (pycnogenol from pine bark and oxykine from melons) SOD, SOD activity in the blood and the immunological content of Mn-SOD both decreased to within the standard values, and the range of variation was drastically narrowed. In general, it is known that oxygen consumption and production of reactive oxygen are accelerated by acute and strenuous exercise.[23,24] On the other hand, as a consequence of habitual and repeated training, sportspeople are subjected to raised plasma SOD activity above the standard level,[25,26] high-density lipoprotein cholesterol (HDL)-cholesterol are high, oxidation of low-density lipoprotein cholesterol (LDL)-cholesterol is suppressed, and there is an increase in antioxidant capacity.[22] In such cases it seems likely that oral ingestion of exogenous SOD extracts is having the effect of suppressing the quantity of SOD synthesized inside the body. Accordingly, it is appropriate to assume that antioxidant capacity is strengthened through the ingestion of antioxidant health foods.

However, the meaning of the fact that the amount of 8-OHdG in the urine showed a tendency to increase due to oral ingestion of SOD is not clear. There are reports to the effect that pycnogenol accelerates the synthesis of antioxidant enzymes inside cells and encourages the elimination of free radicals as scavenger, thus reducing oxidative stress.[27,28] Furthermore, pycnogenol of between 100 and 125 mg/day prevents platelet coagulation reactions and an increase in the concentration of thromboxane A2 due to smoking in the case of smokers.[8,23] On the other hand, it has been shown that pycnogenol has little capacity to prevent DNA damage caused by oxidative stress.[29] Another report[30] also suggested that even if pycnogenol is administered for two weeks at a dosage of 200 mg/day, there is no strengthening vitamin C and antioxidant capacity. Furthermore, when 17 healthy subjects orally ingested oxykine (1000UI-NBT/day) for two weeks, there was no evidence of change in Cu/Zn-SOD activity in the blood, there was a decrease in glutathione peroxidase activity, and an increase in the amount of erythrocyte malondialdehyde (MDA).[31] Another report[32] has shown using oxykine additives to breed mice with insulin non-dependent diabetes results in a three- to four-fold increase in the amount of 8-OHdG in the urine between the fourth and twelfth weeks. In this manner, even if it is assumed that the antioxidant capacity inside the body is raised due to ingestion of SOD, it seems likely that there will be an increase in oxidants (the amount of 8-OHdG in the urine) within a certain length of time thereafter. More serial and frequent measurement of oxidants before and after ingestion and before and after interruption of antioxidant health foods is needed in order to investigate in greater detail changes occurring chronologically.

Smokers and sportspeople who are easily affected by oxidative stress need to ingest sufficient quantities of foods including antioxidant minerals (Cu, Zn, Fe, Se) in order to replenish the coenzymes

of antioxidant enzymes (SOD, glutathione peroxidase, catalase, *etc.*) and antioxidant vitamins (C and E). However, it will be necessary to show further scientific evidences as to whether there is any need for ingestion of antioxidant health foods containing plant SOD.

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