Selenium Level and Glutathione Peroxidase Activity in Plasma, Erythrocytes and Platelets of Healthy Japanese Volunteers

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Summary The purpose of this study was to determine both the selenium (Se) level and glutathione peroxidase (GSH-Px) activity in plasma, erythrocytes and platelets from 51 healthy Japanese individuals. The Se levels (mean $\pm$ SD) of plasma, erythrocytes and platelets were 117.4 ± 15.7 μ g/L, 0.954 ± 0.159 μ g/g hemoglobin, and 4.93 ± 1.52 ng/mg protein, respectively, and GSH-Px activity was 318 ± 50 U/L, 18.0 ± 5.0 U/g hemoglobin, and 0.142 ± 0.035 U/mg protein, respectively. There was a negative correlation between age and the platelet Se level in men ($r = -0.761$, $p < 0.001$), and a positive correlation between the plasma and platelet GSH-Px activities in women ($r = 0.663$, $p < 0.001$).

Key Words plasma, erythrocytes, platelets, selenium, glutathione peroxidase

Selenium (Se), an essential trace element, is a part of glutathione peroxidase (GSH-Px) and other selenoenzymes or selenoproteins, which are involved in the removal of hydrogen peroxide and lipid peroxides produced during oxidative processes in cells (1). The lack of Se in animals and humans causes a decrease in GSH-Px activity, and as a result, lipid peroxides and free radicals may damage cell membranes (2–4).

Various methods for assessing Se status are based on measurements of Se level and/or GSH-Px activity in plasma, erythrocytes and/or platelets. In Japan, although studies have been conducted to determine the Se levels and/or GSH-Px activity in plasma, erythrocytes and/or whole blood from healthy adults (5–13) and TPN patients (5, 14), few studies have dealt with both the Se level and GSH-Px activity in blood components. It is important to determine both parameters in the blood

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components obtained from the same individuals, because this enables evaluation of the overall Se status of individuals, without a confounding influence due to individual variations, differences among sampling times of each blood component, and differences in assay method.

We determined both the Se levels and GSH-Px activity in plasma, erythrocytes and platelets from healthy Japanese individuals and examined the relationships among them in an attempt to obtain basic information regarding Se.

METHODS

Subjects. Fifty-one healthy adults (28 men and 23 women) residing in Shiga, Kyoto and Osaka prefectures took part in this study. The ranges of age for men and women were 25–44 years and 23–51 years, respectively. None showed any abnormalities at the time of physical examination. All subjects gave informed consent before the beginning of the study, and the study protocol was approved by the Ethical Committee of Roussel Morishita Co., Ltd.

Collection of blood components and assay of Se and GSH-Px. Before breakfast, 18 mL of blood was withdrawn from the antecubital vein into a plastic syringe containing 2 mL of 3.2% trisodium citrate solution. The sample was mixed by gentle inversion. The plasma, erythrocytes and platelets were collected by the method of Sando et al (5), and were stored at -80°C until assaying of Se levels and GSH-Px activity.

Each sample (plasma: 250 μL , erythrocyte suspension: 100 μL , platelet suspension: 250 μL) was digested with 1.5 mL of 13 mol/L nitric acid in a heating-block (TPB-62; Advantec Toyo, Tokyo, Japan) at 110°C for 12 h. After cooling at room temperature, 0.5 mL of 9 mol/L perchloric acid was added, and the last digestion step at 120°C for 3 h was carried out. Three milliliters of 6 mol/L hydrochloric acid was added, and Se was reduced from Se^{6+} to Se^{4+} by heating at 120°C for 40 min. This solution was diluted in the same tube with distilled water to 20 mL. The Se level was determined by hydride generation (HFS-2; Hitachi, Tokyo, Japan) using an atomic absorption spectrometer (Z-8100; Hitachi) with a Zeeman-effect background correction system, using a standard curve technique described by Sekine et al (15) and Galgan and Frank (16). To certify the accuracy of Se analysis, we assayed Standard Reference Material bovine serum (SRM1598; National Institute of Standards and Technology, Gaithersburg, MD, USA). The measured Se level in SRM1598 was 42.5 $\mu\text{g/L}$, a value acceptably close to the certified value of 40.0–47.2 $\mu\text{g/L}$.

The GSH-Px activity in plasma, erythrocytes and platelets was determined by the method of Paglia and Valentine (17) with a minor modification. The reaction mixture consisted of 56 mM potassium phosphate buffer (pH 7.0), 1.12 mM EDTA, 1 mM NaN_3 , 0.2 mM NADPH, 1 unit/mL GSSG-reductase, 2 mM GSH and 0.1 mM H_2O_2 , in a final volume of 1 mL. All ingredients except the enzyme source and H_2O_2 were combined at the beginning of each day. A unit was defined as 1 μmol

NADPH oxidized per minute. Blank reactions with enzyme source replaced by distilled water were subtracted from each assay, for nonenzymatic oxidation of NADPH by the peroxides. Protein was measured by the method of Peterson (18).

Statistics. The Se levels and GSH-Px activity for the healthy volunteers were expressed as mean $\pm$ SD. Statistical analyses of data were performed using regression analysis. Statistical significance was set at $p < 0.05$.

RESULTS

As indicated in Table 1, the Se levels in plasma, erythrocytes and platelets were $117.4 \pm 15.7 \mu\text{g/L}$ (analytical range 76.9–149.4), $0.954 \pm 0.159 \mu\text{g/g Hb}$ (analytical range 0.576–1.343) and $4.93 \pm 1.52 \text{ ng/mg protein}$ (analytical range 2.04–7.67), respectively. As indicated in Table 2, the GSH-Px activity levels in plasma, erythrocytes and platelets were $318 \pm 50 \text{ U/L}$ (analytical range 181–410), $18.0 \pm 5.0 \text{ U/g Hb}$ (analytical range 9.9–32.6) and $0.142 \pm 0.035 \text{ U/mg protein}$ (analytical range 0.067–0.214), respectively. There were no significant sex differences in the above Se and GSH-Px values.

Table 3 shows the relation between age and the Se level or GSH-Px activity

Table 1. Se levels of plasma, erythrocytes and platelets in healthy Japanese adults.

		Se level		
	<i>n</i>	Plasma ($\mu\text{g/L}$)	Erythrocytes ($\mu\text{g/g Hb}$)	Platelets (ng/mg protein)
Male	28	120.9 ± 15.6	0.959 ± 0.162	5.21 ± 1.64
Female	23	113.1 ± 15.0	0.949 ± 0.159	4.70 ± 1.42
Total	51	117.4 ± 15.7	0.954 ± 0.159	4.93 ± 1.52

Each value represents mean $\pm$ SD.

Table 2. GSH-Px activities of plasma, erythrocytes and platelets in healthy Japanese adults.

		GSH-Px activity		
	<i>n</i>	Plasma (U/L)	Erythrocytes (U/g Hb)	Platelets (U/mg protein)
Male	28	329 ± 41	17.0 ± 5.0	0.140 ± 0.034
Female	23	304 ± 57	19.2 ± 4.8	0.145 ± 0.036
Total	51	318 ± 50	18.0 ± 5.0	0.142 ± 0.035

Each value represents mean $\pm$ SD. A unit of GSH-Px activity is expressed as $1 \mu\text{mol}$ of NADPH oxidized per minute.

Table 3. Correlation coefficients between age and Se level or GSH-Px activity in each blood component.

	Plasma		Erythrocytes		Platelets	
	Se	GSH-Px	Se	GSH-Px	Se	GSH-Px
Age (Men)	0.000	0.322	0.015	0.559**	-0.761***	0.037
Age (Women)	0.010	0.576**	0.218	-0.067	-0.205	0.407

** $p < 0.01$, *** $p < 0.001$.

Fig. 1. The relationship between age and platelet Se level in male volunteers.

in each blood component. Significant relation to age was seen for the plasma GSH-Px activity in the women and for the erythrocyte GSH-Px activity and platelet Se level in the men. The highest correlation to age was that for the platelet Se level in the men (Fig. 1); the regression equation was $y = -0.413x + 17.368$ ($r = -0.761$, $p < 0.001$). There was no significant correlation between age and any other parameters.

Table 4 summarizes the relationships between the parameters in the three blood components. Significant correlations were observed between the plasma and erythrocyte Se levels and between the plasma and platelet GSH-Px activities in the women, and between the plasma and erythrocyte GSH-Px activities in the men. The strongest relationship was that between the platelet and plasma GSH-Px activities in the women (Fig. 2); the regression equation was $y = 0.410x + 19.660$ ($r = 0.663$, $p < 0.001$). There was no significant correlation between other parameters.

DISCUSSION

The Se levels of plasma and/or erythrocytes in Japanese have been documented in several studies (5, 8, 9, 13), and the data presented here do not contradict those findings. There has been no study on the Se level of platelets in Japanese until now, and our study is the first report. The GSH-Px activities of plasma and erythrocytes

Table 4. Correlation coefficients between parameters in each blood component.

		Se			GSH-Px		
		Plasma	Erythrocytes	Platelets	Plasma	Erythrocytes	Platelets
Se	Plasma	—	0.424*	0.039	0.236	−0.312	0.304
	Erythrocytes	<u>0.372</u>	—	0.243	0.010	0.359	0.243
	Platelets	<u>0.282</u>	<u>−0.229</u>	—	0.066	−0.123	−0.020
GSH-Px	Plasma	<u>0.131</u>	<u>0.143</u>	<u>−0.013</u>	—	−0.253	0.663***
	Erythrocytes	<u>0.101</u>	<u>0.207</u>	<u>−0.053</u>	0.387*	—	−0.262
	Platelets	<u>0.203</u>	<u>0.020</u>	<u>−0.004</u>	<u>0.218</u>	<u>0.148</u>	—

* $p < 0.05$, *** $p < 0.001$.

Non-underlined: women. Underlined: men.

Fig. 2. Relationship between the plasma and platelet GSH-Px activities in female volunteers.

found in our study are similar to those reported by Yoshida (9), Sando et al (5) and Sekine et al (13), although comparisons among these investigations are not straightforward because of the differences in analytical method and the lack of certification of the accuracy of analysis with Standard Reference Material. The platelet GSH-Px activity in Japanese was reported by only Sando et al (5); the value indicated by them is similar to that which we found. Using parametric values obtained in this study, we next tried to clarify the correlations between age and each parameter and between parameters.

Some studies of age-related differences in blood Se level and GSH-Px activity in Japanese adults have been reported. Deguchi and Ogata (8) and Yoshida (19) have indicated that age is a significant factor in the decrease of the serum Se level, but our results do not support this assertion. It is considered difficult to establish whether there is a correlation between age and plasma Se level, because the plasma Se level is liable to be influenced by dietary habits (10, 20). In fact, the correlations reported by Deguchi and Ogata (8) and by Yoshida (19) were weak.

Ito et al (12) reported that there was a weak positive correlation between age and erythrocyte GSH-Px activity ($r=0.2854$, $p<0.01$). We also recognized a positive correlation between these two parameters only in the men, and this may partially support their findings. On the other hand, there has been no report on the relationship between age and platelet Se level or GSH-Px activity in Japanese until now. In this study, we revealed that there was a negative correlation between age and platelet Se level in Japanese men. This is the first report.

The statistically significant correlations between the erythrocyte and plasma Se levels in the women and between the erythrocyte and plasma GSH-Px activities in the men may not be significant, because the correlation coefficients were small ($r<0.5$). That between the platelet and plasma GSH-Px activities in the women may be significant, because the correlation coefficient was large ($r>0.5$). Clear relationships between parameters were not obtained in this study, in spite of the determination by a method without the influence of any of individual variations, differences in sampling times of blood components and differences in assay method. The cause for this is believed to be as follows. Firstly, when using healthy subjects, the ranges of the Se level and GSH-Px activity are very narrow, and therefore analytical error may more markedly affect the experimental results. Secondly, the percentages of Se associated with GSH-Px in plasma, erythrocytes and platelets may be low. Lane et al (21) and Oh et al (22) have reported that the lack of correlation between erythrocyte Se level and GSH-Px activity may be because only 60% of erythrocyte Se is associated with GSH-Px. The percentage of Se associated with GSH-Px is apparently greater in populations with low Se intake, such as in New Zealanders, than in residents of countries where the Se intake is high (23). Accordingly, the confusion of our experimental results may be promoted because Japan is not a low Se status country. Thirdly, the gap of Se levels or GSH-Px activities among blood components is large. In fact, it has been reported that the use of plasma GSH-Px measurement for assessment of Se status seems to be limited in that it comprises less than 5% of the total whole blood GSH-Px activity as measured by the coupled method of Paglia and Valentine using hydrogen peroxide (17).

A clear relationship between age and platelet Se level in the men was found in this study. It has been reported by Perona et al (24) that Se partially regulates the biosynthesis of prostanoids with implications in platelet function. In general, cardiovascular disease is easily contracted as one grows older. Our results suggest that Se is closely related to cardiovascular disease and/or aging. However, we could not reveal by our study alone what this result really means, and further study is required. In addition, a clear relationship between plasma and platelet GSH-Px activities in the women was found in this study. This suggests that plasma GSH-Px activity is useful for assessing the platelet GSH-Px activity, which partially regulates the platelet function. Further study is also required here.

In this study, we demonstrated the Se levels and GSH-Px activities in plasma,

erythrocytes and platelets in healthy Japanese adults. We found that there are some correlations between these parameters and that there is a sex-difference in these correlations. In addition, our results suggest that it is difficult to generalize from the relationships among these parameters in healthy subjects. Further studies of subjects whose Se nutritional status is influenced by external or internal causes such as total parenteral nutrition and elemental diet are needed to obtain comprehensive information regarding Se.

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