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Abstract

Levels of erythrocyte superoxide dismutase (SOD) activity in a sample of Japanese people were determined. Blood samples were taken from new-born infants, preschool children, young and old people who had no apparent diseases and also from three anemic patients. Erythrocyte SOD activities in different age groups had a nearly normal distribution. Females had slightly lower activities than males, although the difference was statistically insignificant. The distributions of SOD activities were 12.6 ± 2.7 (m $\pm$ SD) unit/mg Hb in young people and 11.4 ± 3.0 in old people, indicating that erythrocyte SOD activity falls with aging. Because of low concentration of hemoglobin, SOD activities of old people expressed as unit/ml blood were much lower than in young people. Three anemic patients had slightly lower SOD activity.

KEYWORDS: superoxide dismutase, Japanese erythrocytes, aging process, sex difference, anemic patients, superoxide anion

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LEVELS OF ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY IN JAPANESE PEOPLE

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Abstract. Levels of erythrocyte superoxide dismutase (SOD) activity in a sample of Japanese people were determined. Blood samples were taken from new-born infants, preschool children, young and old people who had no apparent diseases and also from three anemic patients. Erythrocyte SOD activities in different age groups had a nearly normal distribution. Females had slightly lower activities than males, although the difference was statistically insignificant. The distributions of SOD activities were 12.6 ± 2.7 ($m \pm SD$) unit/mg Hb in young people and 11.4 ± 3.0 in old people, indicating that erythrocyte SOD activity falls with aging. Because of low concentration of hemoglobin, SOD activities of old people expressed as unit/ml blood were much lower than in young people. Three anemic patients had slightly lower SOD activity (unit/mg Hb), but the difference was not significant.

Key words: superoxide dismutase, Japanese erythrocytes, aging process, sex difference, anemic patients, superoxide anion

SOD catalyzes the reaction: $O_2^- + O_2^- + 2H \longrightarrow H_2O_2 + O_2$ (1), and the presence of SOD is considered to protect the cells against the toxic effects of superoxide anions generated by a wide variety of biological oxygen reactions (2). It has been reported that the concentration of mercaptane groups in serum decreased with aging (3), indicating that free radical oxidation reactions may occur at a greater rate in elderly people. From this point of view, the levels of erythrocyte SOD, as a scavenger of superoxide anions, was examined in different age groups. SOD is inhibited by some agricultural chemicals such as dimethyldithiocarbamate. Therefore, normal values of SOD are necessary to use as controls for assessing the decrease in SOD activity caused by these agents. Erythrocyte SOD levels in a French population were reported by Michelson *et al.* (4). There are no reports about the Japanese population so far. In a previous report, the authors described how the levels of erythrocyte SOD in Japanese acatalasemias were higher than those of normal control (5), and suggested that erythrocyte SOD activities in the older subjects were lower than those of youths (6). This report describes the distribution of erythrocyte SOD activities found in different

age groups and the difference between males and females expressed both as unit/mg Hb and unit/ml blood. In addition to this, erythrocyte SOD activities of three anemic patients are described.

MATERIALS AND METHODS

Seventy-four blood specimens were obtained for analysis. Blood samples were drawn from the umbilical cord blood of 9 infants born in the Department of Gynecology, Okayama University Medical School Hospital, 11 preschool children aged 4 to 6 undergoing tonsillectomy, 32 young people aged 20 to 35 undergoing regular check-ups at Okayama Public Health Center, 22 old people aged 70 to 100 in a nursing home for aged. They had no apparent diseases. Three blood specimens of anemic patients (mainly hemolytic anemia) were obtained from out-patients of Okayama City Hospital.

Reagents. Xanthine oxidase (purified from milk, Grade IV) and nitroblue-tetrazolium were obtained from the Sigma Chemical Co. The other chemicals were of reagent grade.

Assay of SOD activity. Preparation of crude extract from red blood cells for SOD assay was conducted by the method of Winterbourn *et al.* (7). Red blood cells were washed three times with ice-cold saline and hemolyzed with deionized water. Hemoglobin concentration was measured and adjusted to 10 gm/100 ml. Hemolysate was diluted 8 times with deionized water then added 0.25 volume of ice-cold ethanol, followed by addition of 0.15 volume of chloroform with stirring, then kept in an ice bucket for about 10 min and centrifuged for 10 min at 3,000 rpm. SOD activity was measured by a modification of the method of Winterbourn (7). One unit of this enzyme was defined by the amount of SOD required to get a half maximum inhibition of NBT reduction. The recovery rate of SOD was 93.1 ± 1.6 (m $\pm$ SD) and variation coefficient was 2% (8).

RESULTS

The levels of erythrocyte SOD activity in Japanese people classified by age and sex are shown in Table 1 and the distributions of SOD activities are shown in Fig. 1. Fig. 2 shows a normal probability chart indicating that erythrocyte SOD activity in young and old people and the total population (children, young and old people) and also male or female group of the population had a normal distribution. This is confirmed by the χ^2 test for normal distribution at the 5% level. No significant variations of SOD unit/mg Hb (Table 1A) with respect to sex were observed in 26 males and 39 females taken from all groups. The mean activity, unbiased sample variance, sample variance, upper and lower confidence interval in the different age groups are shown in Table 1B. Table 2 shows the differences between mean activity of each group. Because of low concentration of hemoglobin, erythrocyte SOD activities of young people when expressed as unit/ml blood was about 2.5 times higher than that of old people. The differ-

TABLE 1. MEAN, DEVIATION AND CONFIDENCE INTERVALS (1%) OF ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY (UNIT/MG Hb) IN GROUPS CLASSIFIED BY SEX (A) AND AGE AND SEX (B)

(A) classification by sex

	m ^a	u ^b	s ^c	upp. Ci ^d	low. Ci ^e	n ^f
Male	14.7	3.6	3.5	16.7	12.7	26
Female	14.4	4.2	4.2	16.3	12.5	39

(B) classification by age and sex

	New-born infants	Preschool children			Young people			Old people			Total ^g	Total ^h
		Male	Female	Total	Male	Female	Total	Male	Female	Total		
m ^a	11.7	17.1	18.3	17.9	15.9	15.2	15.5	11.3	11.5	11.4	14.5	14.5
u ^b	2.8	2.4	4.8	4.0	3.2	2.8	3.0	2.1	3.6	3.1	3.9	3.9
s ^c	2.7	2.1	4.4	3.8	3.1	2.7	2.9	2.0	3.5	3.0	3.9	3.9
upp. Ci ^d	13.9	24.2	25.0	21.7	18.5	17.1	16.9	13.9	14.4	13.3	15.8	15.2
low. Ci ^e	9.5	10.0	11.6	14.1	13.3	13.3	14.1	8.7	8.6	9.5	13.2	12.8
n ^f	15	4	7	11	14	18	32	8	14	22	65	80

a mean b unbiased sample variance c sample variance

d upper confidence interval e lower confidence interval f specimen number

g total of 3 groups new born children omitted h total of 4 groups

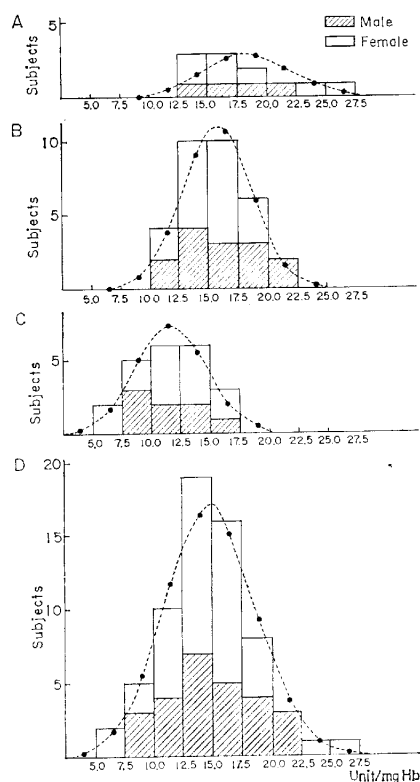


Fig. 1. Distribution of values of erythrocyte superoxide dismutase (SOD) activity in groups classified by age and sex.

(A) SOD activity in preschool children

(B) SOD activity in young people

(C) SOD activity in old people

(D) SOD activity in total of preschool children, young and old people

Dotted lines indicate calculated normal distributions.

ence was statistically significant within 0.1% (Table 3). Three cases of adults with anemia gave a lower mean activity of SOD, when expressed on the basis of ml blood. The decrease, however, was due to lower hemoglobin or erythrocyte content in the blood and was statistically insignificant, when expressed on the basis of hemoglobin.

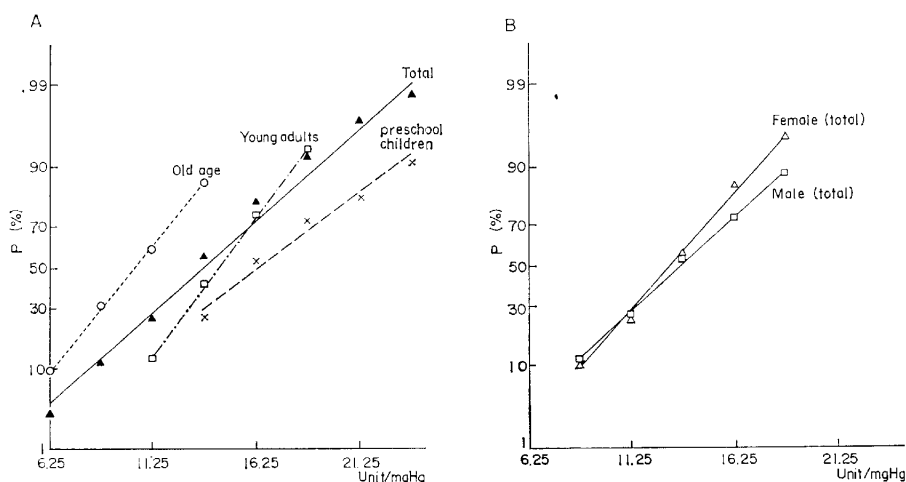


Fig. 2. Normal probability chart on which abscissa shows SOD activity (unit/mg Hb) and ordinate shows distribution function.

(A) groups classified by age

(B) groups classified by sex for the total population

TABLE 2. DIFFERENCES IN MEAN VALUE OF ERYTHROCYTE SOD ACTIVITY AMONG NORMAL SUBJECTS CLASSIFIED BY AGE

	New-born infants	Preschool children	Young people	Old people
New born infants		0.001	0.001	Insignificant
Preschool children			0.050	0.001
Young people				0.001

TABLE 3. MEAN, DEVIATION AND CONFIDENCE INTERVALS (1%) OF ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY (UNIT/ML BLOOD) OF YOUNG AND OLD PEOPLE

	m	u	upp. Ci	low. Ci	n
Young people	2082	698	2389	1775	39
Old people	842	321	304	1172	10

t test: Mean erythrocyte SOD activity between young and old people, $p=0.001$

DISCUSSION

Erythrocyte SOD is extremely important in view of the production of superoxide anions by auto-oxidation of hemoglobin in erythrocyte (9). In our previous report, higher levels of erythrocyte SOD activity in acatalasemic blood than in normal controls were described. In the case of acatalasemic blood, the superoxide anion produced by auto-oxidation of hemoglobin is changed into hydrogen peroxide with SOD and hydrogen peroxide is decomposed with glutathione peroxidase and residual catalase. It seems that aging processes are associated with a falls in SOD activity causing diminished protection against the toxic effects of superoxide anions (10). So, SOD activity could be one of the factors controlling longevity. Recently, Michelson *et al.* (4) reported that the mean SOD activity unit/mg Hb of seven French subjects older than 60 years was lower than that of the total population. Our results which indicate lower level of erythrocyte SOD activity in the old age group agree with their report.

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