Enzyme Activities Involved in the Degradation of Lipid Peroxides in the Senescence-Accelerated Mouse

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Summary The activities of enzymes involved in the degradation of lipid peroxides, viz., glutathione peroxidase, glutathione S-transferase (GST), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G-6-PDH), were measured in the erythrocytes, liver, brain, and heart of male SAM-P/1 mice (accelerated senescence-prone mice) over a period from 1 to 10 months after birth in comparison with those of age-matched male SAM-R/1 mice (accelerated senescence-resistant mice). Among the enzyme activities examined, GR activity in SAM-P/1 mice was markedly lower than that in SAM-R/1 mice in the erythrocytes and all organs tested. GST activity in the liver of the former mice was also lower than that of the latter mice. These results would explain, at least in part, the increased level of lipid peroxides in the former mice as compared with that in the latter. It was noted that G-6-PDH activity increased in the erythrocytes and liver of SAM-P/1 mice.

Key Words: glutathione reductase, lipid peroxide level, senescence-accelerated mice

We reported previously that the serum lipid peroxide level in humans increases with advancing age [1, 2]. An age-dependent increase in the serum lipid peroxide level in rats was also reported [3]. Since the increase in lipid peroxide level in the blood is considered to be causative of age-related degenerative diseases such as atherosclerosis and cataract [4-7], it is important to elucidate the cause of the elevation of lipid peroxide levels in these diseases as well as in aging.

The senescence-accelerated mouse (SAM), developed by Takeda *et al.* [8], is a murine model of aging and seems to be useful for the study of the mechanism of

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aging. In our previous papers [9, 10], we reported that lipid peroxide levels in the serum, liver, and skin of SAM-P/1 mice (accelerated senescence-prone mice) are markedly higher than those of SAM-R/1 mice (accelerated senescence-resistant mice) in the early period of their life, viz., 2–4 months after birth. Since the lipid peroxide level increased prior to the appearance of senescence-associated clinical signs, we suggested that increased lipid peroxides in SAM-P/1 mice probably have a role in initiating and promoting the aging process in these animals.

In the present study, we examined the activities of various enzymes that take part in the degradation of lipid peroxides to approach the cause of an increase in the lipid peroxide level in SAM-P/1 mice.

MATERIALS AND METHODS

Male SAM-P/1 and SAM-R/1 mice were kindly donated by Dr. Toshio Takeda, Kyoto University, and maintained in our institute as described in our previous paper [9]. The number of mice examined was 5 of each strain at each age. Male AKR mice of 3–4 months of age were obtained from Seiwa Experimental Animals, Ltd., Fukuoka. Male ddY mice of 3–4 months of age were obtained from Japan SLC, Inc., Shizuoka. The chemicals used in this study were of reagent grade.

The mice were sacrificed under anesthesia with diethyl ether, and the blood, liver, brain, and heart were taken immediately. The erythrocytes were lysed in ice-cold water after having been washed with physiological saline twice and used for enzyme assays. The tissues were washed with ice-cold physiological saline and homogenized in the saline. In the case of the liver, perfusion with ice-cold physiological saline was carried out before homogenization. The homogenates were centrifuged at $15,000 \times g$ for 20 min, and the supernatant fractions obtained were used for the enzyme assays.

The activity of glutathione peroxidase (GSH-Px) in the red blood cells and tissues was assayed according to the procedure of Awasthi *et al.* [11] with *t*-butyl hydroperoxide as a substrate. The activity of glutathione S-transferase (GST) in the tissues was assayed by the method of Habig *et al.* [12] with 1-chloro-2,4-dinitrobenzene as a substrate; that of glucose-6-phosphate dehydrogenase (G-6-PDH), by the method of Dror *et al.* [13]; and that of glutathione reductase (GR) in the erythrocytes and tissues, by the method of Nichoalds [14]. One unit of activity of each enzyme was defined as the amount of enzyme that catalyzes the conversion of 1 μ mol of substrate into product per min.

Protein concentration of the supernatant fractions of tissue homogenates was measured by the method of Lowry *et al.* [15]. The concentration of hemoglobin (Hb) was measured with a Hemoglobin B-Test kit from Wako Pure Chemical Industries, Ltd., Osaka.

RESULTS

Activities of GSH-Px, GST, and G-6-PDH of SAM

The activity of GSH-Px in the erythrocytes, liver, brain, and heart was not statistically different between SAM-P/1 and SAM-R/1 mice over a period from 1 to 10 months after birth except that the activity of SAM-P/1 mice decreased in the erythrocytes and in the liver at 10 months (data not shown).

The activity of GST in the liver of SAM-P/1 mice was lower than that of SAM-R/1 mice at early and late stages of life, as shown in Table 1; while the activity in the other organs was not different between the two strains of mice.

On the contrary, the activity of G-6-PDH in the erythrocytes and in the liver of SAM-P/1 mice was higher than that of SAM-R/1 mice (Table 2), whereas the

Table 1. Glutathione S-transferase activity in the liver of SAM.

Age (months)	U/mg protein	
	SAM-R/1	SAM-P/1
1	2.35±0.32	1.40±0.14*
2	3.24±0.36	2.68±0.31
3	4.12±0.34	2.06±0.39**
4	3.96±0.47	3.27±0.25
5	3.42±0.39	3.26±0.43
6	3.62±0.91	2.75±0.21
7	2.98±0.73	2.16±0.19
8	2.11±0.40	1.97±0.20
9	2.76±0.18	1.86±0.33*
10	3.99±0.24	1.68±0.29**

Mean±SE is given. $n=5$. Significant difference from values of age-matched SAM-R/1 mice: * $p<0.05$; ** $p<0.01$.

Table 2. Glucose-6-phosphate dehydrogenase of SAM.

Age (months)	Erythrocytes (U/g Hb)		Liver (mU/mg protein)	
	SAM-R/1	SAM-P/1	SAM-R/1	SAM-P/1
1	13.8±0.8	19.2±0.7***	5.05±1.37	9.47±0.83**
2	12.3±0.8	12.4±0.6	4.34±0.47	4.47±0.54
3	13.6±0.8	16.8±0.8**	5.63±0.35	6.84±0.38**
4	12.7±0.3	17.5±0.4***	5.36±0.17	5.84±0.13*
5	11.8±1.7	16.7±0.8**	5.39±0.45	6.30±0.61
6	11.9±1.3	15.8±1.0**	4.79±0.20	5.96±0.52
7	12.8±0.5	15.8±1.1**	6.16±0.98	6.76±1.16
8	12.1±0.3	17.9±0.6***	6.66±0.85	8.66±0.08*
9	14.8±1.2	16.5±0.8	4.69±0.41	6.79±0.35***
10	13.5±0.7	15.6±1.1	6.21±0.56	13.52±1.55***

Mean±SE is given. $n=5$. Significant difference from values of SAM-R/1 mice: * $p<0.1$; ** $p<0.05$; *** $p<0.01$.

Table 3. Glutathione reductase activity in the erythrocytes of SAM.

Age (months)	FAD addition	U/g Hb	
		SAM-R/1	SAM-P/1
1	—	6.07±0.21	6.03±0.23
	+	8.19±0.37	7.72±0.40
2	—	5.81±0.25	4.67±0.40**
	+	8.16±0.27	5.87±0.86**
3	—	6.25±0.14	5.04±0.30**
	+	8.24±0.39	7.15±0.36*
4	—	7.18±0.38	5.91±0.13**
	+	8.63±0.20	7.44±0.28***
5	—	7.86±0.75	5.60±0.32**
	+	9.34±0.85	7.30±0.44*
6	—	6.60±0.27	5.54±0.33**
	+	8.28±0.22	7.11±0.30**
7	—	6.28±0.19	5.79±0.52
	+	8.00±0.52	6.96±0.41
8	—	6.48±0.04	5.42±0.14**
	+	8.03±0.21	7.40±0.35
9	—	6.58±0.76	5.87±0.54
	+	8.55±1.06	7.01±0.34
10	—	6.14±0.47	5.51±0.14
	+	7.99±0.41	7.22±0.39

GR activity was measured with or without the addition of FAD. Mean±SE is given. $n=5$. Significant difference from values of age-matched SAM-R/1 mice: * $p<0.1$; ** $p<0.05$; *** $p<0.01$.

Table 4. Glutathione reductase activity in the liver of SAM.

Age (months)	mU/mg protein	
	SAM-R/1	SAM-P/1
1	107±15	103±7
2	113±10	85±8*
3	126±5	101±4***
4	130±3	102±5***
5	128±6	113±8
6	126±4	99±5***
7	126±9	101±3**
8	114±5	105±7
9	107±6	93±6
10	106±3	99±4

GR activity was measured without the addition of FAD. Mean±SE is given. $n=5$. Significant difference from values of age-matched SAM-R/1 mice: * $p<0.1$; ** $p<0.05$; *** $p<0.01$.

activity in the brain and heart was the same.

GR activity in the erythrocytes of SAM

Table 3 shows the levels of GR activity in the erythrocytes of 1–10-month-old

Table 5. Glutathione reductase activity in the brain of SAM.

Age (months)	mU/mg protein	
	SAM-R/1	SAM-P/1
1	95.3±7.6	71.4±7.2*
2	86.1±9.2	57.3±6.9**
3	100.5±2.6	69.9±3.2***
4	109.7±2.6	70.6±5.5***
5	102.3±2.9	77.5±4.9***
6	95.0±2.7	70.2±3.5***
7	101.5±6.8	65.1±2.1***
8	103.4±3.2	68.8±4.7***
9	100.5±4.2	73.2±2.7***
10	83.3±4.5	69.1±3.7**

GR activity was measured without the addition of FAD. Mean±SE is given. $n=5$. Significant difference from values of age-matched SAM-R/1 mice: * $p<0.1$; ** $p<0.05$; *** $p<0.01$.

Table 6. Glutathione reductase activity in the heart of SAM.

Age (months)	mU/mg protein	
	SAM-R/1	SAM-P/1
1	63.1±13.7	39.8±3.9
2	48.2±3.0	29.2±3.0**
3	55.5±5.0	38.1±1.3**
4	66.1±6.0	41.5±0.7**
5	58.6±4.2	42.8±1.6**
6	58.8±2.1	40.9±2.6**
7	54.1±4.2	39.1±2.1*
8	47.3±2.6	35.2±1.7**
9	53.6±4.0	35.2±2.4**
10	50.9±2.5	37.6±1.6*

GR activity was measured without the addition of FAD. Mean±SE is given. $n=5$. Significant difference from values of age-matched SAM-R/1 mice: * $p<0.05$; ** $p<0.01$.

Table 7. Glutathione reductase activity in 3-4 month-old mice.

	SAM-P/1	AKR	ddY
n	10	6	7
Erythrocytes			
− FAD	5.70±0.27	7.90±0.52**	6.70±0.25**
+ FAD	7.47±0.26	9.52±0.39**	9.50±0.43**
Liver	101±3	130±3**	113±5
Brain	70.2±3.0	106.0±3.8**	80.9±4.1*
Heart	39.8±0.9	55.4±2.0**	38.8±2.1

GR activity in the erythrocytes was measured with or without the addition of FAD, and expressed as U/g Hb. The activity in the organs was measured without the addition of FAD, and expressed as mU/mg protein. Mean±SE is given. Significant difference from values of SAM-P/1 mice: * $p<0.05$; ** $p<0.01$.

SAM-P/1 and SAM-R/1 mice. GR activity in the erythrocytes of SAM-P/1 mice measured without the addition of flavin adenine dinucleotide (FAD) began to be lower than that of age-matched SAM-R/1 mice after 2 months of age; and lower levels continued up to 10 months, the longest time we observed. When the activity of the enzyme was measured with the addition of FAD, the level of SAM-P/1 mice was also lower than that of SAM-R/1 mice. Whereas the ratio of the activity measured with FAD to that without FAD showed no significant difference between SAM-P/1 and SAM-R/1 mice.

GR activity in the liver, brain, and heart of SAM

The levels of GR activity in the liver, brain, and heart of 1–10-month-old SAM-P/1 and SAM-R/1 mice are summarized in Tables 4, 5, and 6, respectively. Since the addition of FAD to the homogenate of each sample did not give any significant difference, we only showed the activity measured without the addition of FAD. In all of these organs of SAM-P/1 mice, the levels tended to be lower than those of SAM-R/1 mice at 1 month of age and became markedly lower after 2–3 months of age. The lower levels continued up to 10 months after birth.

Comparison of GR activity in the erythrocytes, liver, brain, and heart between SAM-P/1 mice and other strains of mice

Since the results described above showed that GR activity in the erythrocytes and all organs tested of SAM-P/1 mice was markedly lower than that of SAM-R/1 mice at 3 and 4 months of age, we compared these data with those of age-matched mice of other strains, viz., AKR mice and ddY mice. The obtained results are shown in Table 7. All the levels of GR activity in SAM-P/1 mice measured were lower than those in AKR mice.

When compared with the levels in ddY mice, the levels in the erythrocytes and brain were lower in SAM-P/1 mice, while the level in the heart was not significantly different.

DISCUSSION

We have been proposing that lipid peroxides increased in the body are a direct cause of age-related diseases and of aging itself. We already found that the level of lipid peroxides in an animal model of aging, SAM-P/1 mice, was higher than the level in the control SAM-R/1 mice.

We suspected that the defect of defense activity against the formation of lipid peroxides might not be primarily important, since the activity of one enzyme in the defense systems, superoxide dismutase, did not change in SAM-P/1 mice as compared with SAM-R/1 mice (data not shown). Our efforts were rather focused on a possible decrease in the rate of decomposition of lipid peroxides, and examined the enzymes which are expected to contribute to the degradation of lipid peroxides in the body.

The present results clearly show that only GR activity in the erythrocytes and important organs of SAM-P/1 mice was lower than that of SAM-R/1 throughout almost all their life. In addition, we found that GR activity of 3–4 month-old SAM-P/1 mice was lower than that of age-matched mice of other strains such as AKR mice, which are thought to be the origin of SAM, and ddY mice. GR requires FAD as a cofactor for its enzyme activity. It is well known that riboflavin deficiency brings about a decrease in GR activity and in turn a decrease in the level of the reduced form of glutathione, resulting in a decrease in lipid peroxide-degrading activity in tissues. Accordingly, it is important to examine the reason for the decrease in GR activity in SAM-P/1 mice. As shown in Table 3, the decrease in GR activity in the erythrocytes of SAM-P/1 mice was not recovered much by the addition of FAD to the reaction system. In the case of organs, GR activities measured with or without the addition of FAD were nearly the same. Moreover, we observed that levels of total flavins and FAD in the blood and in the liver of 3–4 month-old SAM-P/1 mice were the same as those of SAM-R/1 mice (data not shown). These results indicate that the decrease in GR activity in SAM-P/1 mice is not ascribable to riboflavin deficiency, but most probably to a decrease in biosynthesis of GR protein or to production of a less active form of GR.

The decrease in the activities of GR and GST in SAM-P/1 mice reported in this paper would explain, at least in part, the increased level of lipid peroxides in this animal model of aging. The increase in the activity of G-6-PDH seems to be a reflection of the enhancement of lipid peroxidation in this animal as in the case of rats fed tocopherol-stripped corn oil [16].

Considering the data presented already [17, 18] that GR activity is decreased in old animals, the role of GR in the aging process should be further investigated.

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