Age-related Changes in Glutathione Concentration, Glutathione Peroxidase, Glutathione-S-Transferase, and Superoxide Dismutase Activities in Senescence Accelerated Mice

Puming He, Sakiyo Yamaoka-Koseki, and Kyoden Yasumoto*

Research Institute for Food Science, Kyoto University, Gokasho, Uji, Kyoto 611, Japan
Received September 6, 1993

Age-associated changes in several antioxidative factors were studied in blood and liver from mice prone to accelerated senescence aged 0.7 to 1.4 years and mice resistant to accelerated senescence aged 1.0 to 2.1 years. In both strains, activities of hepatic glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) decreased significantly with age, and the activity of superoxide dismutase (SOD), and the concentration of glutathione (GSH) in liver and blood cells tended to decrease with age. The GSH concentration in the plasma and blood cells, and the hepatic GST and SOD activities were significantly lower in the mice prone to senescence than other strain, but the hepatic GSH concentration and GSH-Px activity were similar in the two strains. These results suggest that GSH and hepatic antioxidative enzymes are associated to some extent with senescence accelerated senescence.

Free radicals are important causes of aging.1,2 They are produced in cells through both enzymatic and nonenzymatic reactions. Free radicals from oxygen cause oxidative damage to proteins, lipids, and nucleic acids.3–5 Glutathione (GSH) and a variety of enzymes are part of the intracellular defense mechanism against the toxicity of oxygen-reactive species6; the enzymes include superoxide dismutase (SOD) and GSH-dependent enzymes such as glutathione peroxidase (GSH-Px), GSH reductase, and GSH-S-transferase (GST). GSH levels decrease with age in mosquitoes,7 mice,8,9 and human.10,11

Several groups have studied changes in the activities of antioxidative enzymes in a different of species and tissues. Most of the results are conflicting or equivocal. For example, in studies of the effect of aging on liver antioxidative enzymes such as GSH-Px, SOD, and catalse in rodents, some have shown an age-associated increase,12,13 others have shown an age-associated decrease,14–17 and still others have shown changes not related to age.1,8,18

These conflicting reports prompted us to study the changes in GSH, GSH-Px, and other antioxidative factors during the life span of mice that can be caused as a model of aging. Senescence-accelerated mice (SAM), derived from AKR/J mice, were established by Takeda et al.20,21 The “mice prone to accelerated senescence,” one strain of SAM, develop and mature normally and then undergo accelerated senescence with shorter life span, compared with the “mice resistant to accelerated senescence,” the other strain of SAM, which show normal aging. The mice prone to senescence have been used as a model of aging in studies of senile amyloidosis,22 senile cataracts,23,24 degenerative arthritis, and senile osteoporosis.25 This study examined the relationship between age and antioxidative factors (GSH concentration in the blood and liver, and specific activities of GSH-Px, GST, and SOD in the liver) with one subline of the “mice prone to accelerated senescence,” SAMPI/Fky, and with one substrain of the “mice resistant to accelerated senescence,” SAMR1/Fky.

Materials and Methods

Animals and diets. Mice of the SAMPI/Fky and SAMR1/Fky strains were reared in our laboratory at 23±1°C with a 12-h light-dark cycle. Animals were given commercial feed (CE-2, Clea Japan, Inc.) and deionized water ad libitum.

Collection of samples. Animals, 0.7 to 1.4 years-old for SAMPI/Fky and 1.0 to 2.1 years-old for SAMR1/Fky, were anesthetized with ether and killed by decapitation. Trunk blood was collected in an Eppendorf tube with about 1 mg of powdered EDTA 2H2O. The liver was removed within 5 min after the decapitation, and stored at −20°C until analysis.

Assay of GSH in blood and liver. Plasma was separated from blood by centrifugation and was deproteinized with 30% perchloric acid. The extract was neutralized by the addition of potassium carbonate.26 The separated blood cells were hemolyzed by the addition of acetic acid, and GSH was extracted with 5-sulfosalicylic acid.27 A portion of liver was homogenized in 0.6 N perchloric acid. The supernatant obtained by centrifugation (1000 g, 15 min) was neutralized with potassium carbonate and then assayed for GSH. Another portion of the liver was homogenized in 50 mM potassium-sodium phosphate buffer (pH 7.0). The supernatant after centrifugation (1000 g, 5 min) was centrifuged again (100,000 g, for 1 h). The enzyme activities in the supernatant were assayed after this centrifugation.

The method described by Owens and Belcher28 and modified by Tietze29 was used for enzymatic assay of the GSH concentration with a slight modification. In brief, the following reagents were mixed in a cuvette: 50 mM sodium phosphate buffer containing 1 mM EDTA, pH 7.1, 2.5 ml; 20 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) in 100 mM sodium phosphate buffer (pH 7.0), 30 µl; and sample solution or the standard (0 to 20 µg of GSH/ml), 0.2 ml. After the cuvette was kept at 37°C for 2 min, the reaction was started by the addition of 1.5 units/ml glutathione reductase (0.1 ml) and 4 mg/ml NADPH (0.1 ml). The rate of DTNB reduction, as monitored by the increase in absorbance at 412 nm, was proportional to the concentration of GSH in the cuvette over the concentration ranges found in this study. The GSH concentration in the samples was calculated from a calibration curve generated with standard GSH.

Abbreviations and strains: SAM, senescence-accelerated mouse; SAMR1/Fky, mice resistant to accelerated senescence; SAMPI/Fky, mice prone to accelerated senescence; GSH, glutathione; GSH-Px, glutathione peroxidase; GST, glutathione-S-transferase; SOD, superoxide dismutase; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid.

* To whom correspondence should be addressed.

Table Glutathione Concentrations in Blood and Liver and Liver Antioxidative Enzyme Activities in SAMP1/Fky and SAMR1/Fky

<table>
<thead>
<tr>
<th>Glutathione concentration</th>
<th>Plasma (μg/ml)</th>
<th>Blood cells (mg/g Hb)</th>
<th>Liver (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMP1/Fky</td>
<td>4.73 ± 0.43</td>
<td>1.65 ± 0.06</td>
<td>7.79 ± 0.30</td>
</tr>
<tr>
<td>SAMP1/Fky</td>
<td>3.74 ± 0.18b</td>
<td>1.52 ± 0.03b</td>
<td>7.32 ± 0.21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hepatic antioxidative enzyme activities</th>
<th>GSH-Px (IU/g)</th>
<th>GST (IU/g)</th>
<th>SOD (IU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMP1/Fky</td>
<td>68.2 ± 2.7</td>
<td>423 ± 41</td>
<td>9.18 ± 0.29</td>
</tr>
<tr>
<td>SAMP1/Fky</td>
<td>67.9 ± 3.0</td>
<td>315 ± 11b</td>
<td>7.82 ± 0.27b</td>
</tr>
</tbody>
</table>

*Values are represented as means ± SEM for 15 SAMP1/Fky 0.7 to 1.4 years-old and 15 SAMP1/Fky 1.0 to 2.1 years-old. Results are those in Figs. 1–6.

**Significantly different at p < 0.05 when compared with the results for SAMP1/Fky.

Assay of liver enzyme activities. Liver GSH-Px activity was assayed by the coupled-enzyme procedure with tert-butyl hydroperoxide as the substrate332 with previously noted modifications.333 SOD activity was measured in terms of the ability of the enzyme to prevent the reduction of cytochrome c by O2·− generated by the xanthine-xanthine oxidase system.333,334 GST activity was assayed at 25°C in 100 mM sodium phosphate buffer containing 1 mM EDTA, pH 6.5, with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH as the substrates as described by Habig et al.333 The increase in the absorbance at 340 nm was recorded. One unit of GST activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of product per minute under the assay conditions, and was calculated with the molar absorption coefficient of 9.6 mM−1 cm−1.

Assay of plasma protein and blood hemoglobin. The protein in the liver and plasma was measured as described by Lowry et al.34 and the hemoglobin concentration in the blood cells and plasma caused by the contamination of hemolysis of blood cells was measured as previously described with a kit (Hemoglobin-Test-Wako, code 279-10701, Wako Pure Chemical Ind.).311

Statistics. The results in the Table are means ± SEM, and statistical analysis was done by Student’s t-test with a significance level of p < 0.05. Linear regression analysis was used to assess the relationship between antioxidative factors and animals age. The correlation coefficient, r was analyzed by Student’s t-test, in which the t distribution was calculated by the formula:

\[ t = \frac{r \sqrt{n-2}}{\sqrt{1-r^2}} \]

where n was the sample number and the degree of freedom was n−2.

Results

Glutathione concentration in blood and liver

The GSH concentration tended to decrease with age in blood cells and liver from both strains of mice (Figs. 1 and 2). The decrease in livers from SAMR1/Fky with age was significant. In plasma from SAMP1/Fky, changes were not associated with age; in plasma from SAMR1/Fky, the concentration seemed to increase with age (Fig. 3).

Liver antioxidative enzyme activities

Hepatic GSH-Px activity decreased significantly with age in both strains of mice (Fig. 4). The specific activity of GST tended to decrease with age in SAMP1/Fky and decreased significantly with age in SAMR1/Fky (Fig. 5). Hepatic SOD activity tended to decrease with age in both strains of mice (Fig. 6).
Age-associated Changes in Antioxidative Factors

![Graph of Glutathione Peroxidase Activity vs. Age](image1)

Fig. 4. Glutathione Peroxidase Activity in Liver of SAMP1/Fky and SAMR1/Fky at Different Ages.

For symbols, see the legend of Figs. 1 and 2.

![Graph of Glutathione-S-Transferase Activity vs. Age](image2)

Fig. 5. Glutathione-S-Transferase Activity in Liver of SAMP1/Fky and SAMR1/Fky at Different Ages.

For symbols, see the legend of Figs. 1 and 2.

![Graph of Hepatic Superoxide Dismutase Activity vs. Age](image3)

Fig. 6. Changes in Hepatic Superoxide Dismutase Activity in SAMP1/Fky and SAMR1/Fky at Different Ages.

For symbols, see the legend of Fig. 1.

Discussion

Our finding of a decrease in GSH with age in both strains of mice was consistent with earlier findings that GSH decreases with age in erythrocytes, liver, kidneys, and heart. Such a decrease would weaken the antioxidative defense system, and may accelerate senescence, as suggested previously. This interpretation is consistent with the hypothesis of Harman that aging proceeds because of the destructive action of free radicals. There was much individual variation in the plasma GSH concentration. However, the range was 1/100 to 1/50 that in blood cells, so plasma GSH seems to reflect least the senescence process in mice (Fig. 3).

Hepatic antioxidative enzyme activities decreased with age. The decreases with age were consistent with earlier observations that GSH-Px activity is 25% to 53% lower in liver of 36-month-old mice than in 10-month-old mice, that SOD activity is 16.1 versus 7.0 IU/mg hepatic protein for 6-month-old rats compared with those 26-month-old rats, and that hepatic glutathione-S-transferase activity decreases linearly with age. The mRNA levels of GSH-Px and SOD in hepatocytes also decrease with age. These observations, taken together, suggest that GSH and the antioxidant enzymes GSH-Px, GST, and SOD are associated to some extent with senescence. Tolmasoff et al. has postulated that the longevity of mammals is dependent on antioxidant defense mechanisms, including that involving SOD. The results from this study is evidence for the suggestion.

Senescence in SAMP1/Fky does not occur during development; but does in an accelerated manner following a normal development. The median survival time of SAMP1/Fky, 1.05 years, is significantly shorter than that of SAMR1/Fky mice, 1.3 years. For these reasons, we assessed changes in the antioxidative defense mechanism when the SAMP1/Fky were 0.7 to 1.4 years old, and when the SAMR1/Fky were 1.0 to 2.1 years old. These periods were approximately from 2/3 to 4/3 of the median survival time of SAM. We assumed that strain-specific basic activities could be represented by the means of the activities over these periods. The mean of the GSH concentration and the activities of hepatic antioxidative enzymes shown in Figs. 1–6 are compared in Table. That the GSH concentration in the plasma and blood cells and the activities of hepatic GST and SOD were higher in SAMR1/Fky than in SAMP1/Fky suggests that these higher levels of antioxidative factors contributes to the longer life span of SAMR1/Fky. This conclusion is consistent with the observation that SOD and catalase are correlated with the life span of mice, rats, guinea pigs, rabbits, pigs, and cattle.

Acknowledgments. The authors thank Professor T. Takeda for supplying the mating pairs of senescence-accelerated mice. We also thank Mr. H. Naka for his help in the experiments and for taking care of animals. This research was supported by a Grant-in-Aid for Scientific Research (01480061) from the Ministry of Education, Science, and Culture of Japan.

References


