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Original Contribution

ALTERATIONS OF ANTIOXIDANT ENZYMES AND OXIDATIVE DAMAGE TO MACROMOLECULES IN DIFFERENT ORGANS OF RATS DURING AGING

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Abstract—Oxygen free radicals have been hypothesized to play an important role in the aging process. To investigate the correlation between the oxidative stress and aging, we have determined the levels of oxidative protein damage and lipid peroxidation in the brain and liver, and activities of antioxidant enzymes in the brain, liver, heart, kidney, and serum from the Fisher 344 rats at ages of 1, 6, 12, 18, and 24 months. The results showed that the level of oxidative protein damage (measured as carbonyl content) in the brain and liver was significantly higher in older animals than in young animals. No statistical difference was observed in the lipid peroxidation of the liver and brain between young and old animals. The activities of antioxidant enzymes in most tissues displayed an age-dependent decline. Superoxide dismutases in the heart, kidney, and serum, glutathione peroxidase activities in the serum and kidney, and catalase activities in the brain, liver, and kidney, significantly decreased during aging. Cytochrome *c* oxidase, an enzyme involved in electron transport in mitochondria, initially increased, but subsequently decreased in the aged brain, whereas no significant alteration was observed in the liver mitochondrial antioxidant enzymes. The present studies suggest that the accumulation of oxidized proteins during aging is most likely to be linked with an age-related decline of antioxidant enzyme activities, whereas lipid peroxidation is less sensitive to predict the aging process. © 1998 Elsevier Science Inc.

Keywords—Antioxidant enzymes, Lipid peroxidation, Protein oxidation, Aging, Fisher 344 rats, Free radical

INTRODUCTION

Aging is the progressive deterioration in physiological functions and metabolic processes. In recent years, the reactive oxygen species (ROS) have become an active field in aging research because of their potential involvement in many degenerative processes.[1] It is well known that utilization of oxygen represents an efficient mechanism for aerobic organisms to generate energy, but ROS, as the by-products during this process and other unfavorable events, are also produced within the biological system.[2] These ROS are highly reactive and capable of damaging many biological macromolecules such as DNA, RNA, protein, and lipids.[1–3] A particular consequence of this ROS-mediated attack is the accumulation of oxidatively damaged macromolecules, which may lead to genetic mutation and cellular senescence, if not timely removed in vivo.[4]

To protect cells against oxidative damage by oxidants produced during the oxygen metabolism, an antioxidant system has presumably evolved in aerobic organisms.[5] Antioxidant enzymes constitute an important defense system to clear up the detrimental ROS in vivo. Superoxide dismutases (SOD) including MnSOD in mitochondria and CuZnSOD in cytosol rapidly convert superoxide anion ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2). The later can be converted to more harmful hydroxy radicals (HO·) in the presence of transition metals such as iron and copper. Catalase (CAT) and glutathione peroxidase (GPx) can decompose H_2O_2 to water. Any factors that undermine the activities of antioxidant enzymes may lead to accumulation of ROS and subsequently oxidative damage to biological macromolecules.[5]

Mitochondrial electron transport chain is widely

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viewed as the main locus in the cell for the generation of $O_2^{\bullet -}$.[6] Cytochrome *c* oxidase plays an impotent role in the mitochondrial respiratory chain that converts molecular oxygen into water.[7] Cytochrome *c* oxidase activity has been found to decline with aging.[8,9] Correlated with the decline of this enzyme is the concomitant increase in the flux of mitochondrial $O_2^{\bullet -}$ and H_2O_2 generation in both insects and mammals.[10,11] To confirm the correlation between oxidative stress and the aging process, we have determined oxidatively modified lipids and proteins and the activities of antioxidant enzymes, namely, SOD, GPx, and CAT, as well as cytochrome *c* oxidase activities in different organs of rats during the aging processes.

MATERIALS AND METHODS

Reagents and chemicals

Reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione reductase (GSSG-R), H_2O_2 stock solution, cumene hydroperoxide, 2,4-dinitrophenylhydrazine (DNPH), malondialdehyde (MDA), thiobarbituric acid (TBA), 1,2,3-trihydroxynezene (pyrogallol), sodium azide, and cytochrome *c* were purchased from Sigma Chemical (ST. Louis, MO) and BCA protein assay kit from Pierce (Rockford, IL).

Animals

Male Fisher 344 rats of different ages were purchased from The Toxicological Center Program (Little Rock, AR), sponsored by National Institute on Aging. Twelve rats for each age group were maintained in the animal facility under standard conditions (12-h light/12-h dark cycle, humidity at 50 ± 15%, temperature 22 ± 2°C, and 12 air changes/h) for 1 week to recover from the shipment stress. Diet and water were given ad lib as instructed by the provider.

Preparation of serum

Rats of each age were sacrificed by decapitation. Prior to sacrifice, rats were anesthetized with carbon dioxide, and blood was collected by heart puncture into anticoagulant-free test tubes. Blood samples were kept at the room temperature for 30 min and then centrifuged at 2,000 rpm for 10 min. The supernatant was collected as serum and stored at −80°C for assay of antioxidant enzymes. The protein concentration was determined by the Pierce BCA protein assay kit.

Isolation of mitochondria

Mitochondria in the liver were isolated using the method described by Aprille et al.[12] Tissues were homogenized in the ice-cold buffer containing 0.25 M sucrose, 1 mM EDTA, and 1 mM Tris-HCl, pH 7.4. The homogenate was first centrifuged at 600 × *g* for 10 min at 4°C, and the supernatant fractions were collected and further centrifuged at 8,000 × *g* for 20 min to pellet mitochondria. After washing with 0.25 M sucrose buffer containing 1 mM EDTA and 1 mM Tris-HCl, pH 7.4, mitochondrial pellets were resuspended in 0.25 M sucrose buffer containing 1 mM Tris-HCl, pH 7.4 and stored at −80°C. Mitochondria in the brain were isolated using the method described by Satav et al.[13] Tissues were homogenized in the buffer containing 0.3 M mannitol, 0.1 mM EDTA, pH 7.4. Homogenates were centrifuged at 600 × *g* for 10 min at 4°C. The supernatant fractions were collected and followed by centrifugation at 10,000 × *g* for 10 min 4°C to obtain the brain mitochondria. The mitochondrial pellets were washed three times with 0.25 M sucrose buffer containing 0.1 mM EDTA, pH 7.4, resuspended in 0.25 M sucrose buffer, pH 7.4 and stored at −80°C for further analyses.

Assay for protein carbonyl

Tissues were homogenized in PBS, pH 7.4, and debris removed by centrifugation at 12,000 × *g* for 10 min. Supernatant was recovered and the protein concentration was determined using Pierce BCA protein assay kit. The protein carbonyl contents in brain and liver were analyzed by 2,4-dinitrophenylhydrazine (DNPH) method as described by Levine et al.[14] Briefly, 1 ml of tissue supernatant containing 0.5 mg protein was pipetted into the tubes, to which 4.0 ml of DNPH in 2.5 M HCl was added. The blank was made by adding with 2.5 M HCl only. Samples were incubated at the room temperature for 1 h. Then, protein was precipitated by adding 5 ml of 20% trichloroacetic acid and washed three times with 4 ml of ethanol:ethyl acetate (1:1). Precipitated protein was redissolved in 2.0 ml of 6 M guanidine HCl, 20 mM potassium phosphate, pH 6.5, and insoluble substance removed by centrifugation. Carbonyl content was calculated from the maximum absorbance (360–370 nm) using a molar absorption coefficient of 22,000 $M^{-1}cm^{-1}$. The results were expressed as nmol carbonyl per mg protein.

Assay for lipid peroxidation

The lipid peroxides were estimated in the brain and liver homogenates using a modified thiobarbituric acid (TBA) test described by Ohkawa et al.[15] with a slight modification. Briefly, 0.1 ml of homogenate of brain and liver was added to the test tube containing 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution, pH 3.5, and 1.5

ml of 0.8% TBA solution. The mixture was diluted to 4.0 ml with distilled water and heated at 95°C for 60 min. After cooling on ice, the samples were extracted with 4 ml of the mixture of *n*-butanol and pyridine (15:1, v/v). After centrifugation at 3,000 rpm for 10 min, the organic phase was collected and the absorbance measured at a wavelength of 532 nm. The results were expressed as nmol MDA per mg protein.

Assay for total SOD, MnSOD, and CuZnSOD

Total SOD activity was determined from its ability to inhibit the auto-oxidation of pyrogallol according to Maestro et al.[16] The reaction mixture (1 ml) consisted of 50 mM Tris(hyroxymethyl)aminomethane (pH 8.2), 1 mM diethylenetriamine pentaacetic acid, and 20–50 μl of samples. The reaction was initiated by the addition of pyrogallol (final concentration of 0.2 mM), and the absorbance measured kinetically at 420 nm (25°C) for 3 min. MnSOD activity was determined under the same conditions of total SOD assay with addition of 0.1 M NaCN to the assay buffer for 15 min to inhibit CuZn SOD activity. CuZnSOD activity was obtained by subtracting the MnSOD activity from the activity. The final results were expressed as μg SOD per gram of wet tissue.

Assay for GPx and CAT

GPx activity was assayed as described by Mohandas et al.[17] The reaction mixtures (1 ml) containing 50 mM potassium phosphate (pH 7.0), 1 mM sodium azide, 2 mM GSH, 0.2 mM NADPH, 1 unit/ml GSSG-R, 1.5 mM cumene hydroperoxide, and 20–100 μl of samples were incubated at 25°C for 5 min. The reaction was initiated by the addition of cumene hydroperoxide. The kinetic change was spectrophotometrically recorded at 340 nm (25°C) for 3 min. The GPx activity was expressed as a unit, which is defined as the unit (μmol of oxidized NADPH per min) per gram of wet tissue. Catalase (CAT) activities were assayed using the method described by Claiborne.[18] The reaction mixture (1 ml) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H_2O_2, and a 20–50 μl sample. The reaction was initiated by the addition of H_2O_2, and absorbance changes were measured at 240 nm (25°C) for 30 s. The molar extinction coefficient for H_2O_2 is 43.6 $M^{-1}cm^{-1}$. The CAT activity was expressed as the unit that is defined as μmol of H_2O_2 consumed per min per gram of wet tissue.

Measurement of cytochrome c *oxidase activity*

Cytochrome *c* oxidase activity was determined using the method described by Errede et al.[19] The reaction mixture consisted of 40 mM Tris, 100 mM cacodylate, 30 μM ferrocytochrome *c* and 5 μg of mitochondrial protein. The rate of oxidation of cytochrome *c* was measured by recording the absorbance alteration at 550 nm using an extinction coefficient $\Delta\epsilon_{\text{red-ox}} = 18.7$ $mM^{-1} \cdot cm^{-1}$. The enzyme activity was expressed as cytochrome *c* oxidase nmol/mg protein.

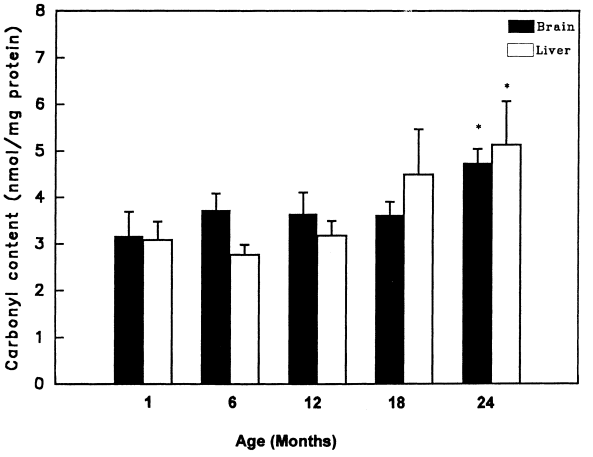

Fig. 1. Protein carbonyl content in the brain and liver from rats of different ages. Data are the mean ± SE from 12 rats per group and expressed as nmol of carbonyl group/mg protein. Statistical analysis for the brain: $*p < .05$ compared to 1-, 6-, 12-, and 18-month-old rats. Fro liver: $*p < .05$ compared to 1-, 6-, and 12-month-old rats.

Statistical analysis

Data were expressed as means ± SE. The two-tail Student's *t*-test was used to assess the significance with $p < .05$ being considered statistically significant.

RESULTS

Protein oxidation in the liver and brain during aging

Carbonyl content was measured as a marker of protein oxidation. As shown in Fig. 1, protein carbonyl content began to increase in the brain of rats at the age of 6 months and in the liver at age of 18 months. However, only the 24-month-old rats showed a statistically significant elevation of the protein carbonyl content in both brain and liver with a 50% increase in brain ($p < .05$) and 66% increase in liver ($p < .05$) when compared with rats at age of 6 months.

Lipid peroxidation in the liver and brain during aging

Figure 2 shows that the lipid peroxidation of brain and liver, measured as the formation of MDA, did not show significant differences among the rats of different ages

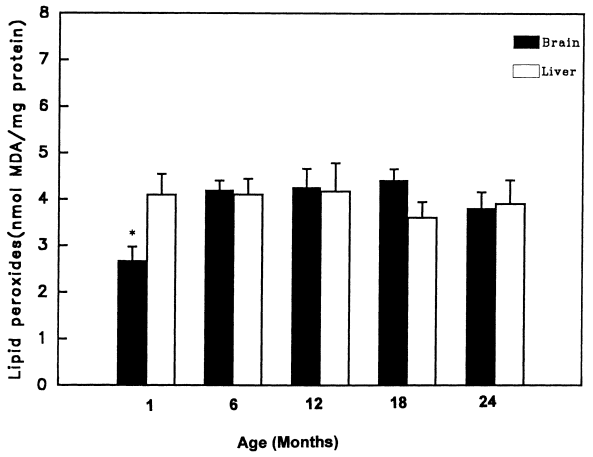

Fig. 2. The levels of lipid peroxides in the brain and liver homogenates from rats of different ages. Data are the mean ± SE from 12 rats per group and expressed as nmol of MDA/mg protein. *$p < .05$ compared to other age groups.

except that the lipid peroxidation in the brain of 1-month-old rats was significantly lower than those of 6-, 12-, 18-, and 24-month-old rats ($p < .05$).

SOD activities in the serum and tissues during aging

To examine the impact of aging on SOD enzymes, we measured total SOD, MnSOD, and CuZnSOD activities in the serum, brain, liver, heart, and kidney. Figure 3 shows that total SOD activity in the serum markedly decreased in an age-dependent fashion. For example, the activity of SOD of 24-month-old rats was only 11 and 17% those of 1- and 6-month-old rats ($p < .01$), respectively. Tissue distribution of SOD showed that the liver had the highest activities of total SOD and CuZnSOD, whereas the most predominant MnSOD activities were observed in the heart (Fig. 4A–C). In addition, SOD activities were substantially lower in the brain than in other tissues. Rats at age of 1 month showed the lowest activities of total SOD, MnSOD, and CuZnSOD in all tissues, which may imply the immaturity of the antioxidant defense system or inadequate induction of antioxidant enzymes due to insufficient exposure to prooxidant conditions. From 6 months old to 24 months old, total

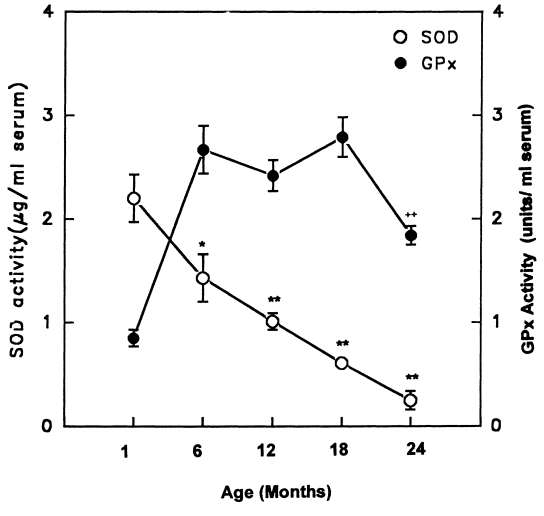

Fig. 3. Activities of SOD and GPx in the serum from rats of different ages. The values are mean ± SE from 12 rats per group. Statistical analysis for SOD: *$p < .05$ and **$p < .01$ vs. 1-month-old rats. Statistical analysis for GPx: $^{++}p < .01$ vs. 6-month-old rats.

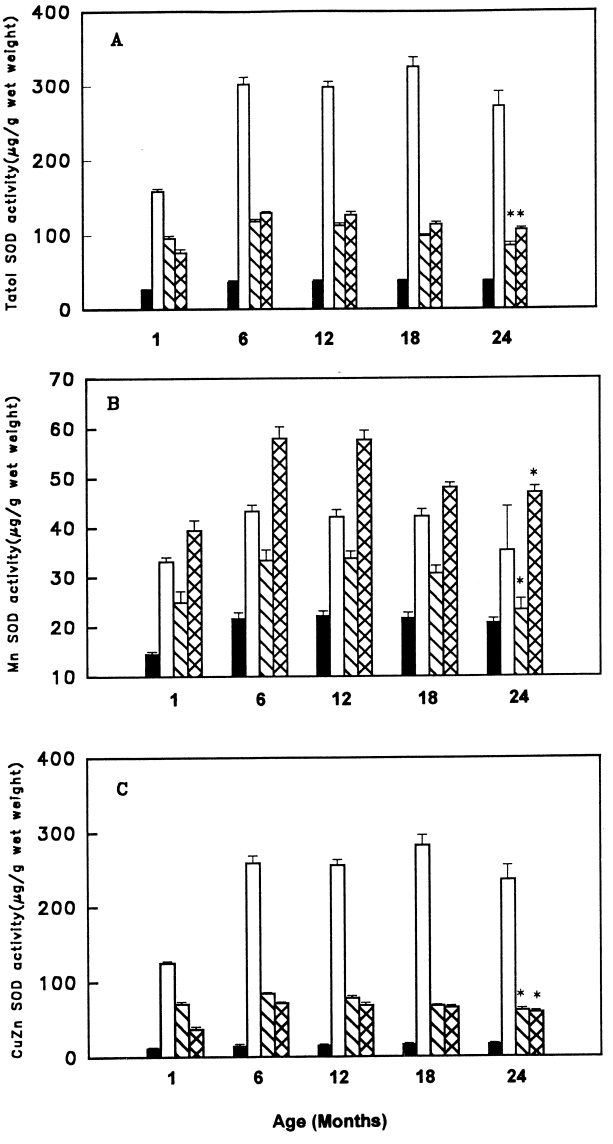

Fig. 4. Activities of antioxidant enzyme activities in various organs from rats of different ages. Data are the mean ± SE from 12 rats per group. (A) total SOD, (B) MnSOD, and (C) CuZnSOD. *p < .05 compared to 6-month-old rats. Legends: filled columns, brain; open columns, liver; slanted rule column, kidney; and crosshatched columns, heart.

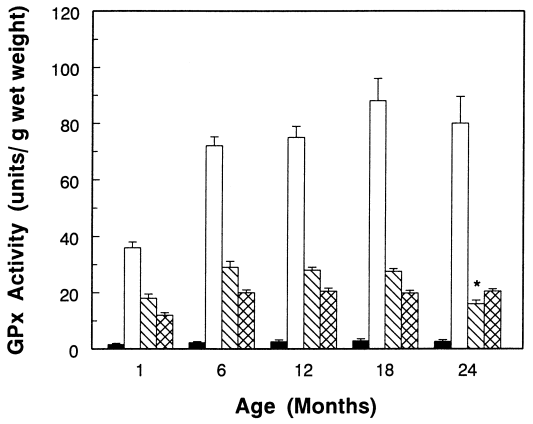

Fig. 5. GPx activities in various organs from rats of different ages. Data are the mean ± SE from 12 rats per group. *$p < .05$ compared to 6-month-old rats. Legends: solid columns, brain; open columns, liver; slanted rule columns, kidney; and crosshatched columns, heart.

SOD, MnSOD, and CuZnSOD activities in the heart and kidney showed an age-dependent decrease, with a statistically significant decline in 24-month-old compared to 6-month-old rats ($p < .05$). However, no age-related changes in total SOD, MnSOD, and CuZnSOD activities were observed in liver and brain (Fig. 4A–C).

GPx activities in serum and tissues during aging

GPx activity in the serum of 1-month-old rats was significantly lower ($p < .01$) than those of other age groups (Fig. 3). The activity of GPx in serum initially increased, remained stable from age of 6 to 18 months, and then significantly declined in 24-month-old rats ($p < .01$). GPx activities from various organs showed that only GPx activity in the kidneys of 24-month-old rats was significantly lower than those of 6-, 12-, and 18-month-old rats ($p < .05$), and no age-related alterations in GPx activities were observed in the brain, liver, and heart (Fig. 5). In addition, the GPx activity was markedly lower in the brain than other tissues, and was significantly lower in all tested organs of 1-month-old rats ($p < .01$).

Cat activities in tissues during aging

As shown in Table 1, CAT activities of the brain, liver, and kidney of 24-month-old rats were significantly lower than those of 6- and 18-month-old rats. In addition, the brain exhibited the lowest CAT activity among all tested tissues. Similar to GPx, the CAT activity in the liver and kidney of 1-month-old rats was significantly lower than those of other age groups ($p < .01$).

Cytochrome c *oxidase activity in the brain and liver mitochondria during aging*

Figure 6 shows that the activity of cytochrome *c* oxidase in mitochondria of the brain and liver from rats of different ages. Cytochrome *c* oxidase activity of liver in old-age groups was significantly lower than that in 1-month-old rats ($p < .01$), and no significant differences were observed between 6-, 12-, 18-, and 24-month-old rats (Fig. 6A). The activity of cytochrome *c* oxidase in brain was lower in 1-month-old than other age groups ($p < .01$), increased in 6-month-old and then decreased in 24-month-old (Fig. 6B). A statistically significant decline of cytochrome *c* oxidase activity in cerebral mitochondria was observed between 6-month-old and 24-month-old rats ($p < .05$).

SOD, GPx, and CAT activities in the brain and liver mitochondria during aging

Antioxidant enzyme activities in mitochondria showed a different pattern than in tissue homogenate. The SOD activity of liver mitochondria increased from 1-month-old to 6-month-old rats and then gradually declined with age (Fig. 7A). Liver mitochondrial SOD activities were significantly lower in 18- and 24-month-old rats than in 6-month-old rats ($p < .05$). However, no significant difference in brain mitochondrial SOD was observed between young and old rats (Fig. 7A). Neither did the mitochondrial GPx of liver and brain show a significant difference between young and old rats (Fig. 7B). In contrast, the mitochondrial CAT activity in liver

Table 1. Age-Related Changes in Catalase Activities in Tissues from Rats of Different Ages*

	Catalase Activities (Units/g Wet Tissue)				
Age (Months)	1	6	12	18	24
Brain	1.6 ± 0.2	2.2 ± 0.1	2.4 ± 0.1	2.2 ± 0.1	1.7 ± 0.1†
Liver	40.4 ± 1.3	92.1 ± 4.8	90.7 ± 4.5	92.9 ± 7.0	62.5 ± 8.1‡
Kidney	14.5 ± 0.7	15.3 ± 0.4	13.5 ± 0.7	12.0 ± 0.8	7.0 ± 0.8‡

* Data are the mean ± SE from 12 rats for each group.
† $p < .05$ vs. 6-, 12-, and 18-month-old rats.
‡ $p < .01$ vs. 6-, 12-, and 18-month-old rats.

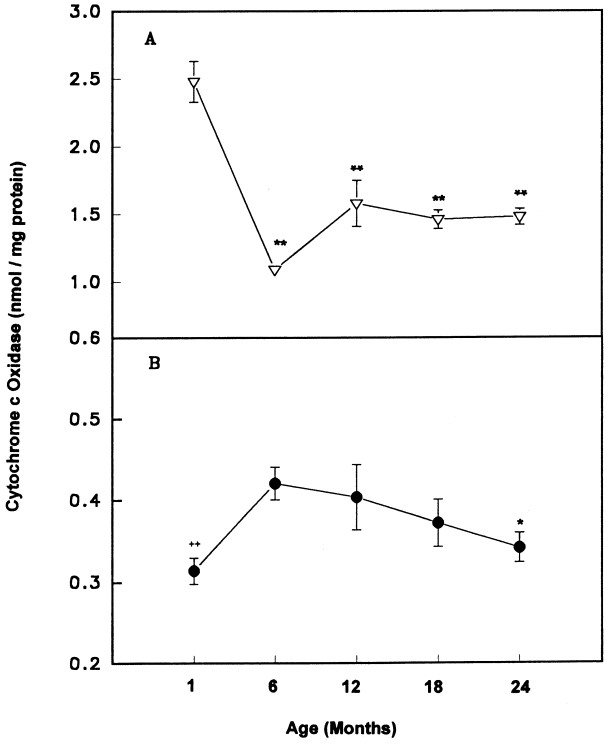

Fig. 6. Cytochrome *c* oxidase activities in mitochondria of the liver and brain from rats of different ages. Data are the mean ± SE from 12 rats. (A) Liver mitochondria: cytochrome *c* oxidase activity in old age groups is lower than that in 1-month-old rats (**$p < .01$). (B) Brain mitochondria: cytochrome *c* oxidase activity is lower in 1-month-old rats than other old groups ($^{++}p < .01$) and lower in 24-month-old than in 6-month-old rats (*$p < .05$).

was higher in 1-month-old rats, but sharply declined in an age-dependent fashion (Fig. 7C).

DISCUSSION

ROS are usually derived from abnormally interrupted metabolism of oxygen and thought to play an important role in oxidative damage to biological macromolecules. The mitochondrial electron transport chain is widely viewed as the main site in the cell for $O_2^{\bullet -}$ and H_2O_2 generation. The reduction of molecular oxygen in the cells involved the cytochrome a/a_3 complex or cytochrome oxidase, which catalyzes the transfer of four electrons from reduced cytochrome *c* to molecular oxygen. Although molecular oxygen is a good electron acceptor, a partial or incomplete transfer provides a flux of oxygen radical from mitochondria, subsequently leading to an excessive formation of ROS in the biological system. Increasing evidence indicates that oxygen radical production in the cell increases with age in mammals and insects.[20,21] The age-related increase in production of prooxidants may be derived from the membrane damage by $O_2^{\bullet -}$ and H_2O_2.[21] This may lead to accumulation of

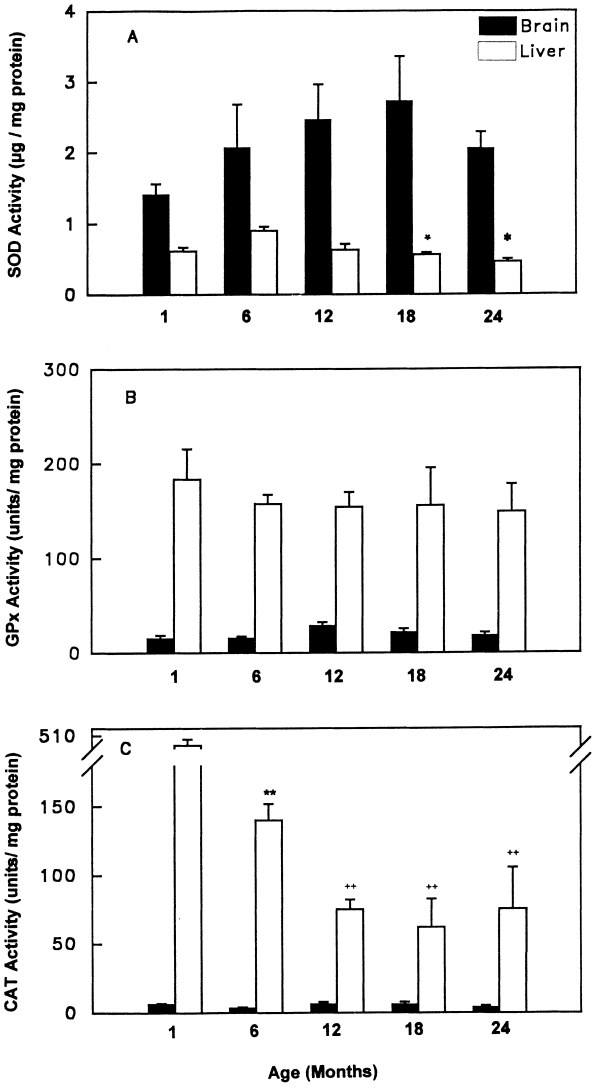

Fig. 7. Antioxidant enzyme activities of in the liver and brain mitochondria from rats of different ages. Each value is mean ± SE from 12 rats. (A) MnSOD, *$p < .05$ vs. 6-month-old rats; (B) GPx and (C) CAT, **$p < .01$ vs. 1-month-old rats and $^{++}p < .01$ vs. 6-month-old rats.

oxidatively damaged macromolecules, including DNA, RNA, lipids, and crucial enzyme proteins in senescent cells.

In the present study, significantly elevated protein carbonyl contents were observed in the brain and liver of old rats. Our findings are in agreement with the animal and human studies from other investigators.[22,23] It is well known that proteins are susceptible to damage by ROS in vitro and in vivo and oxidative modification of proteins may lead to the structural alternation and functional inactivation of many enzyme proteins.[24] Normally, oxidatively modified proteins are degraded more rapidly than native proteins by proteolytic system,[25] which is proposed as a secondary free radical defense system.[26]

Thus, the age-related accumulation of oxidatively modified proteins is due to either excessive oxidation of proteins or decreased capacity to clear up oxidatively damaged proteins. In addition, there is evidence that free radical-mediated mixed-function oxidation play an important role in the formation of protein carbonyl in vitro and in vivo.[27,28]

Although no significant difference in the liver cytochrome *c* oxidase activity was observed between young and old rats, the cytochrome *c* oxidase activity of brain was significantly decreased in the old rats compared to young rats. The decline of cytochrome *c* oxidase may result in the partial blockage of electron flow, which in turn increases potentials of some electron carriers, and consequently accelerates in the rate of its autoxidation and formation of $O_2^{\bullet -}$.[29] Nohl et al.[21] have observed that the age-related production of $O_2^{\bullet -}$ was organ dependent, highest in the brain, and lowest in the liver. The decline in activity of cytochrome *c* oxidase of old brain may lead to the excessive generation of ROS.

Antioxidant enzymes are considered to be a primary defense that prevents biological macromolecules from oxidative damage. SODs rapidly convert $O_2^{\bullet -}$ to less dangerous H_2O_2, which is further degraded by CAT and GPx to water. Thus, the steady-state level of antioxidant enzymes during aging may protect some important tissues against free radical-mediated damage. Our results showed that the activities of SODs (total SOD, MnSOD and CuZnSOD) in heart and kidney and GPx activities in the kidney were significantly decreased during aging. However, SOD activities in the brain and liver, and GPx activities in the brain, liver, and heart remained unchanged with age. The serum showed an age-related decrease in total SOD activity. Also, the GPx activity of serum from 24-month-old rats was significantly lower than other age groups except 1-month-old rats. These observations suggest that overall antioxidant enzyme status in vivo could be undermined during aging and is best manifested by serum enzyme activities, although some antioxidant enzyme activities remain unchanged in certain tissues.

CAT activity consistently decreases in both tissues and mitochondria as a function of age (Table 1 and Fig. 7). CAT catalyzes the decomposition of H_2O_2 to produce water and molecular oxygen, and plays a major role in protecting cells against oxidative damage.[31] Decreased CAT activity may compromise the overall antioxidant enzyme defense system. GPx in tissues appeared to be less affected by age. It is known that GPx per se is not an efficient H_2O_2 decomposer and high levels of H_2O_2 occur in GPx-sufficient, but CAT-depleted cells.[31,32] Therefore, the compromised CAT activities may significantly impair the capacity of the antioxidant enzyme defense system. It should be noted that the activities of antioxidant enzymes in the brain were markedly lower than those of other tissues. The brain is an aerobic organ that has one of the highest oxygen consumption rates on the basis of the weight. Thus, the brain may be the tissue susceptible to oxidative damage by free radicals because the brain is relatively deficient in antioxidant enzyme defense against ROS. In the mitochondria, MnSOD and CAT activities significantly decreased with age, although the activities of GPx remained unchanged during aging. This suggests that mitochondria in the brain and liver are relatively deficient in antioxidant enzymes, and are more susceptible to oxidative stress during aging because mitochondria are a major site of $O_2^{\bullet -}$ and H_2O_2 generation.

Despite the increased protein carbonyl content in the brain and liver from old rats, no difference was observed in the lipid peroxidation in the brain and liver between young and old rats. There are a wide range of conflicting results with lipid peroxidation during aging. A possible explanation for these discrepancies may due to the differences in the animal model, tissues examined, and assay methods employed.[30] It should be emphasied that the assay we used for MDA is not sepcific, and other TBA reactants such as a trace amount of protein and DNA may be included. Based on the fact that there is a great diversity of lipid peroxidation during aging, we measured MDA levels in multiple tissues using two different methods and we obtained the similar results, suggesting that accumulation of lipid peroxidation may be minimal in the aged tissues. Presumably, oxidatively damaged lipid is subject to rapid degradation.[33]

Taken together, we have demonstrated that the oxidatively damaged proteins accumulated as a function of age. Oxidative protein damage may cause the decline in enzyme activities of the antioxidant system and probably the electron transporting system, subsequently leading to excessive ROS generation in the biological system. In the present studies, oxidative damage to lipids is not evident among different age groups, which may contribute to the high turnover rate of lipid peroxidation products and efficient repair mechanisms in vivo.

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ABBREVIATIONS

CAT—catalase
DNPH—2,4-dinitrophenylhydrazine
GPx—glutathione peroxidase
GSH—reduced glutathione
GSSG—oxidized glutathione
H_2O_2—hydrogen peroxide
HO·—hydroxyl radicals
MDA—malondialdehyde
$O_2^{\cdot -}$—superoxide anion radical
ROS—reactive oxygen species
SOD—superoxide dismutase
TBA—thiobarbituric acid