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Age-associated changes in oxidative damage and the activity of antioxidant enzymes in rats with inherited overgeneration of free radicals

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Abstract

Reactive oxygen species have been hypothesized to play an important role in the process of aging. To investigate the correlation between oxidative stress and accumulation of protein and DNA damage, we have compared the age-dependent levels of protein carbonyl groups and the activities of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in cytosol and mitochondrial extracts from liver cells of Wistar and OXYS rats. The latter strain is characterized by increased sensitivity to free radicals. Faster age-dependent increase in the level of protein carbonyl groups was found in OXYS as compared with Wistar rats. A complicated enzyme-specific pattern of age-dependent changes in the activities of antioxidant enzymes was observed. Long-term uptake of dietary supplements Mirtilene forte (extract from the fruits of *Vaccinium myrtillus* L.) or Adrusen zinco (vitamin E complex with zinc, copper, selenium and ω-3 polyunsaturated fatty acids) sharply decreased the level of protein oxidation in cytosol and mitochondrial extracts of hepatocytes of Wistar and of OXYS rats. Both dietary supplements increased the activity of catalase in the liver mitochondria of OXYS rats. Our results are in agreement with the shorter life-span of OXYS and with the mitochondrial theory of aging, which postulates that accumulation of DNA and protein lesions leads to mitochondrial dysfunction and accelerates the process of aging.

Keywords: OXYS rats aging • nuclear and mitochondrial extracts • protein carbonyl groups • antioxidant enzymes.

Introduction

All higher organisms generate energy by aerobic respiration, a process that involves a stepwise four-electron reduction of molecular oxygen to water. The partially reduced species that are produced as intermediates and by-products of aerobic respiration, including $^{\cdot}O_2^-$, H_2O_2, and $^{\cdot}OH$, are potent oxidants attacking different cellular components including DNA of mitochondria and nuclei [1]. These oxidants also appear in cells through exposure to ionizing radiation and other agents that generate free radicals. A major target of reactive oxy-

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gen species (ROS) is the cellular genome, and oxidative stress can lead to DNA damage resulting in numerous genotoxic adducts, DNA strand breaks and mutations. Oxidative damage to the cell is ongoing and has been regarded as a significant factor in carcinogenesis and aging [1–7]. Therefore, special attention had been given to understanding the mechanism of oxidative DNA damage and repair. Overgeneration of free radicals in animals can also be accompanied by modification of lipids and proteins [8–10]. Mitochondrial proteins in human muscle are particularly susceptible to free radical-induced oxidative damage [11].

Inherited overgeneration of free radicals in animals is accompanied by a number of morbid conditions resembling human aging-associated degenerative diseases such as cataracts, cardiomyopathy, carcinogenesis and others, as well as short lifespan and low fertility [1, 7, 12, 13]. By selecting rats for susceptibility to the cataractogenic effect of a galactose-rich diet and siblings mating of the highly susceptible animals, an inbred strain (OXYS) was developed from the Wistar stock [14]. The main characteristics of the OXYS strain are inherited overproduction of free radicals or increased sensitivity to free radicals, high levels of lipid peroxidation, protein oxidation and DNA rearrangements, and conditions resembling the human degenerative diseases mentioned above [14]. Thus, the OXYS strain could be validated as a good model to study human degenerative diseases and the mechanism of oxidative protein and DNA damage and repair in mammals.

Antioxidant enzymes such as superoxide dismutases (SOD), catalases (CAT), and glutathione peroxidases (GPx), represent critical defense mechanisms for preventing oxidative modifications of DNA, proteins and lipids [15, 16]. Therefore, we have compared age-dependent levels of several antioxidant enzymes in the liver cells of OXYS and Wistar rats.

Material and methods

Materials and chemicals

Reagents used in this work were obtained from Merck and Sigma Chemical Co.

Animals

Male white Wistar and OXYS rats [14] at 3, 6, 12 and 15 months of age were used in this study. The second group of Wistar and OXYS rats (12-months-old), consisting of three subgroups (each of 13 animals), was used for three dietary treatment. Diet 1 was the basal diet used for the control group of rats. Diets 2 and 3 were the same as the basal diet with the addition of 230 mg Mirtilene forte/kg weight or 1 g Adrusen zinco/kg weight, respectively. Mirtilene forte is an extract of bilberry shrub and is normalized to 25% anthocyanosides. Adrusen zinco contains 340 mg of proteins, 260 mg of carbohydrates, 350 mg of lipids including polyunsaturated fatty acids, 23 mg of zinc, 1 mg of copper, 88 mg of selenium and 24 mg of vitamin E per gram.

The animals were housed in colonies at the animal breeding facility of SD RAS Institute of Cytology and Genetics (Novosibirsk, Russia) under standard conditions. The rats were decapitated, and their livers were immediately removed and processed.

Preparation of mitochondrial extracts

Rat liver mitochondria were isolated using a combination of differential and gradient centrifugation in sucrose as described in [17]. All procedures were carried out at 4°C. Briefly, fresh livers were perfused by 150 mM KCl, minced and homogenized in a buffer containing 10 mM Tris-HCl (pH 7.4), 25 mM KCl, 1 mM EDTA, 2 mM DTT, 250 mM sucrose, 0.15 mM spermine, 0.75 mM spermidine, and 1 mM phenylmethanesulfonyl fluoride. Unbroken cells and nuclei were pelleted at 800×*g* for 10 min. The supernatant containing mitochondria was centrifuged at 14,000×g for 10 min. The mitochondrial pellet was resuspended and washed twice in a buffer containing 10 mM Tris-HCl (pH 7.4), 1 mM EDTA and 250 mM sucrose. The mitochondrial extracts were prepared by suspending the mitochondria in the lysis buffer containing 20 mM HEPES-NaOH (pH 7.5), 0.4 M NaCl, 5 mM EDTA, 3 mM DTT, 5% glycerol, and 0.3% Nonidet P-40 and clarified by centrifugation for 3 h at 40,000×*g* and then for 1 h at 100,000×g. The nucleic acids content was followed by measuring the $A_{260/A280}$ ratio, which was usually ~1.0, confirming the complete nucleic acids removal. Protein concentrations were determined according to Lowry using bovine serum albumin as a standard [18].

Protein carbonyl content measurements

Protein carbonyl content was measured by forming labeled protein hydrazone derivatives with 2,4-dinitrophenylhydrazide which then were quantified spectrophotometrically [19] from the absorbance at 370 nm (extinction coefficient, 22×10^3 l·mol^{-1} cm^{-1}). The concentration of carbonyl groups was expressed as nmol carbonyl per mg protein.

Measurements of MnSOD and Cu,ZnSOD

SOD activity in the extracts was assayed by two methods. In one method, the SOD activity was measured after electrophoresis of corresponding extracts (20–60 μg total protein) in 5.5% polyacrylamide gel as described previously [20]. Briefly, the gel was incubated in dark in a solution of tetrazole blue and then of riboflavin; a photograph of the gel then revealed a sharp colorless band of MnSOD or Cu,ZnSOD on a blue background. Relative activities of SOD were determined by scanning using the Gel Documentation system (Ultra-Violet Products). MnSOD and Cu,ZnSOD activities were also quantified using a xanthine/xanthine oxidase-mediated ferricytochrome *c* reduction assay [21]. Samples (40 μl) were added to 550 μl of the reaction mixture containing 0.025 μmol of xanthine in 0.1 mM NaOH, and 0.01 μmol of cytochrome *c* in 50 mM phosphate buffer (pH 7.8), supplemented with 0.1 mM EDTA and 10 mM sodium azide. The reaction was initiated by adding 10 μl of the xanthine oxidase solution (0.5 U/ml in 0.1 mM EDTA). The final reaction mixture (0.6 ml) for the assay of MnSOD activity contained 2 mM KCN. The absorbance change was monitored for 3 min at 25°C. SOD activity was calculated from the absorbance at 550 nm using a concurrently run standard curve and expressed as units of activity per mg protein [46]. One unit of SOD activity is defined as the amount of enzyme that inhibits the rate of cytochrome *c* reduction under the conditions specified by 50% [21].

Measurement of CAT

CAT activity was assayed as described in [22]. Decomposition of H_2O_2 (10 mM) was followed directly by the decrease in absorbance at 240 nm (ε_{240} = 39.4 l·mol^{-1}·cm^{-1}). The difference in absorbance (ΔA_{240}) per second was calculated. The CAT activity was expressed as *k* (s–1) per mg/ml of the analyzed sample of protein [22].

Measurement of GPx

GPx activity was assayed as described in [23]. The extracts (50 μl) were added to 250 μl of 0.1 M potassium phosphate buffer (pH 7.0). Then 50 μl of freshly prepared glutathione reductase (2.4 U/ml) in 0.1 M potassium phosphate buffer, pH 7.0, and 50 μl of 10 mM reduced glutathione were added. After incubation for 10 min at 37°C, 50 μl of 1.5 mM solution of NADPH in 0.1% $NaHCO_3$ was added and the incubation was continued for 3 min at 25°C. The reaction was initiated by the addition of 50 μl of 1.5 mM H_2O_2. The absorbance change was followed at 340 nm (25°C) for 4 min. The GPx activity was expressed as units (μmol of oxidized NADPH; e340 = 6200 l·mol^{-1}·cm^{-1}) per min.

Statistical analysis.

The results are reported as the mean and the standard deviation of at least 3 different experiments for each nuclear or mitochondrial extract or cytosol sample of each animal, averaged over at least 5 different animals. The number of preparations assayed for each age is shown in the Tables. The differences between samples were analyzed by the Student's *t*-test, $p < 0.05$ was considered statistically significant.

Results

In the present study, we have examined the age-dependent oxidative damage to proteins in liver cell cytosol (microsomal fraction) and mitochondria of OXYS and Wistar rats. We have determined the carbonyl group content in proteins of OXYS and Wistar rats at 3, 6, and 12 months of age. Table 1 demonstrates that the oxidative damage to the proteins in the mitochondria is ~1.5–2-fold higher than in the cytosol. The protein carbonyl content in cytosol and mitochondrial extracts of OXYS and Wistar rats changed in a complicated way. In the liver cytosol from both strains, it increased ~1.2-fold at 6 months compared to 3 months, and then decreased at 12 months. On the contrary, oxidation

Table 1 Age-associated changes in concentration of carbonyl groups in the liver cytosol (microsomal fraction) and the mitochondrial extracts of OXYS and Wistar rats

Age, months	Number of Wistar / OXYS rats	Protein carbonyl groups in Wistar extracts, nmol/mg	Protein carbonyl groups in OXYS extracts, nmol/mg	Ratio of carbonyls in OXYS to Wistar*
Protein carbonyls in mitochondrial extracts				
3	5/5	10.34 ± 0.85	7.04 ± 0.56	**0.68**
6	5/5	4.34 ± 0.19	4.34 ± 0.24	1.00
12	9/10	6.13 ± 0.29	7.62 ± 0.72	**1.24**
Protein carbonyls in cytosol extracts				
3	5/5	3.68 ± 0.22	3.82 ± 0.30	1.04
6	5/5	4.42 ± 0.28	4.52 ± 0.25	1.02
12	6/6	3.00 ± 0.11	3.69 ± 0.26	**1.23**

*Statistically significant ($p < 0.05$) differences in the parameters determined for OXYS and Wistar rats are given in boldface.

of mitochondrial proteins decreased between 3 and 6 months by a factor of 1.6–2.4 and then increased in both strains at 12 months. Interestingly, nearly the same reproducible difference (1.23-fold) between the two strains in the protein carbonyl content in both cytosolic fraction and mitochondrial extracts was observed in 12-month-old animals.

Under normal circumstances, nuclear and mitochondrial oxygen radicals may be detoxified by SOD activities, decreasing the levels of protein, lipid, and DNA modification. In order to estimate possible differences in such detoxification in OXYS and Wistar rats, we have compared SOD activities in the mitochondrial extracts and cytosol fraction. SOD activity of the nuclear fraction extracts was very low, probably due to leakage of SOD from nuclei to cytosol during cell fractionation. There was no detectable difference in the Cu,ZnSOD activity between mitochondrial extracts of Wistar and OXYS rats of the same age (data not shown). Age-dependent differences in the Cu,ZnSOD activity was found in the cytosol fraction of liver cells. Interestingly, this activity was higher in 3-months-old OXYS rats as compared with Wistar, but was higher in the Wistar rats at 6 and 12 months; statistically significant difference (1.37-fold) was revealed only for 6-months-old animals.

Table 2 demonstrates nearly a linear age-dependent decrease in the level of MnSOD activity in the mitochondria of Wistar and OXYS rats. This activity was 1.24- to 1.43-fold (statistically significantly) higher in OXYS than in Wistar rats at 3 to 6 months of age and then became higher in Wistar rats at 12 months, but the latter difference was not statistically significant.

Other antioxidant enzymes such as CAT and GPx also can play critical roles in cell protection against oxidative stress [15, 16]. We have compared age-dependent levels of these antioxidant enzymes in the liver cells of OXYS and Wistar rats. Table 3 demonstrates nearly the same level of the CAT activity in the cytosol of rats at 3 and 6 months and its remarkable increase at 12 months of age. There was no statistically significant difference in this activity between Wistar and OXYS rats. The age-associated profile of the CAT activity in the mitochondrial extracts was quite different

Table 2 Age-associated changes in the MnSOD and Cu,ZnSOD activities in the cytosol and the mitochondrial extracts of OXYS and Wistar hepatocytes

Age, months	Number of Wistar /OXYS rats	SOD activity in Wistar extracts, U/mg	SOD activity in OXYS extracts, U/mg	Ratio of OXYS to Wistar activity*
MnSOD in mitochondrial extracts				
3	9/7	15.40 ± 1.44	19.15 ± 1.39	**1.24**
6	5/5	8.80 ± 0.79	12.61 ± 1.25	**1.43**
12	9/13	12.38 ± 1.61	10.91 ± 0.92	0.88
Cu,ZnSOD in cytosol extracts				
3	8/9	41.08 ± 3.56	52.96 ± 5.80	**1.29**
6	5/5	59.13 ± 3.86	42.98 ± 4.13	**0.73**
12	10/11	74.29 ± 7.75	69.87 ± 8.70	0.94

*Statistically significant ($p < 0.05$) differences in the parameters determined for OXYS and Wistar rats are given in boldface.

Table 3 Age-associated changes in the CAT activity in the cytosol and the mitochondrial extracts of OXYS and Wistar hepatocytes

Age, months	Number of Wistar /OXYS rats	CAT activity in Wistar extracts, (mg/ml)/s	CAT activity in OXYS extracts, (mg/ml)/s	Ratio of OXYS to Wistar activity*
CAT in mitochondrial extracts				
3	12/12	0.93 ± 0.10	0.98 ± 0.10	1.05
6	5/5	0.46 ± 0.06	0.43 ± 0.06	0.93
12	7/7	0.36 ± 0.07	0.62 ± 0.08	**1.72**
CAT in cytosol extracts				
3	11/11	0.46 ± 0.07	0.47 ± 0.06	1.02
6	5/5	0.44 ± 0.05	0.31 ± 0.04	**0.70**
12	12/12	0.80 ± 0.06	0.59 ± 0.05	**0.74**

*Statistically significant ($p < 0.05$) differences in the parameters determined for OXYS and Wistar rats are given in boldface.

Table 4 Age-associated changes in the GPx activity in the cytosol and the mitochondrial extracts of OXYS and Wistar hepatocytes

Age, months	Number of Wistar /OXYS rats	GPx activity in Wistar extracts, units/min	GPx activity in OXYS extracts, units/min	Ratio of OXYS to Wistar activity
GP in mitochondrial extracts				
3	12/12	0.97 ± 0.04	1.17 ± 0.14	1.21
6	5/5	1.19 ± 0.16	1.19 ± 0.12	1.00
12	7/7	0.70 ± 0.08	0.70 ± 0.11	1.00
GP in cytosol extracts				
3	9/9	1.02 ± 0.11	0.94 ± 0.14	0.92
6	5/5	1.10 ± 0.10	1.08 ± 0.15	0.98
12	7/7	1.53 ± 0.13	1.42 ± 0.18	0.93

Table 5 Effect of Adrusen zinco (OXYS(Zn) and Mirtilene forte (OXYS(M) on the level of protein carbonyl groups in the cytosol and mitochondria of 12-month-old OXYS and control Wistar rats

Animals	Protein carbonyl groups in OXYS extracts, nmol/mg	Number of Wistar / OXYS rats	Ratio of carbonyls in OXYS(Zn) or OXYS(M) to OXYS *
Protein carbonyls in mitochondrial extracts			
OXYS(M)	7.13 ± 0,88 (0.39)	10/10	0.94
OXYS(Zn)	5.64 ± 0,99 (0.44)	10/10	**0.74**
OXYS	7.62 ± 1,64 (0.72)	10/10	1,0
Wistar	6.13 ± 0,67 (0.29)	9/9	–
Protein carbonyls in cytosol extracts			
OXYS(M)	2.34 ± 0,38 (0.16)	7/7	**0.63**
OXYS(Zn)	2.10 ± 0,38 (0.15)	6/6	**0.57**
OXYS	3.69 ± 0,66 (0.26)	6/6	1.0
Wistar	3.00 ± 0,28 (0.11)	6/6	–

*Statistically significant ($p < 0.05$) differences in the parameters determined for OXYS and Wistar rats are given in boldface.

from that in the cytosol fraction. The mitochondrial extracts of OXYS demonstrated a high level of the activity at 3 months that significantly decreased in the 6-months-old rats. Interestingly, the CAT activity changed little from 6 to 12 months in Wistar rats, but there was a significant increase in

Table 6 Effect of Adrusen zinco (OXYS(Zn) and Mirtilene forte (OXYS(M) on the level of catalase activity (CAT) in the cytosol and mitochondria of 12-month-old OXYS rats and control Wistar rats

Animals	CAT activity in extracts, (mg/ml)/s	Number of Wistar /OXYS rats	Ratio of OXYS(Zn) or OXYS(M) to OXYS CAT activity *
CAT in mitochondrial extracts			
OXYS(M)	1.13 ± 0,27 (0.12)	10/10	**1.82**
OXYS(Zn)	0.78 ± 0,16 (0.07)	10/10	1.26
S	0.62 ± 0,20 (0.08)	7/7	1.0
W	0.36 ± 0,17 (0.07)	7/7	
CAT in cytosol extracts			
OXYS(M)	0.71 ± 0,07 (0.03)	10/10	1.22
OXYS(Zn)	0.69 ± 0,16 (0.07)	10/10	1.17
S	0.59 ± 0,16 (0.07)	12/12	1.0
W	0.80 ± 0,14 (0.06)	12/12	

*Statistically significant ($p < 0.05$) differences in the parameters determined for OXYS and Wistar rats are given in boldface.

this activity in the mitochondrial extracts of OXYS rats. Finally, the CAT activity at 12 months was ~1.7-fold (statistically significantly) higher in OXYS than in Wistar rats.

Table 4 shows the data on age-associated changes in GPx activity in the cytosol fraction and in the mitochondrial extracts from liver cells of Wistar and OXYS rats. Age-dependent modifications of this activity in cytosol and in mitochondrial extracts varied, but there was no pronounced difference in this activity between Wistar and OXYS rats for both analyzed fractions. Interestingly, both CAT and GPx activities in the cytosol and the mitochondrial extracts demonstrated an inverse relationship: the age-dependent increase in these activities in the cytosol was accompanied by their decrease in the mitochondria.

Adrusen zinco had a significant effect on the levels of protein oxidation products in cytosol and mitochondria (Table 5). The mitochondria of the control group of rats had a 1.4-fold ($p < 0.05$) higher level of protein carbonyls than those of the supplemented group. Similarly to the mitochondrial extracts, the protein carbonyl content in cytosolic fraction of OXYS rats fed Adrusen zinco decreased ~2-fold ($p < 0.001$) and became lower than in the Wistar control group. We have found that treatment with Mirtilene forte was also effective in preventing the age-related increase in oxidative damage to cytosolic proteins (Table 5). At the same time, there was no significant effect on carbonyl content in the mitochondria of OXYS rats treated with Mirtilene forte.

Table 6 illustrates effects of Adrusen zinco and Mirtilene forte on the level of liver CAT activity. Mirtilene forte significantly (1.8-fold) enhanced the CAT activity in the mitochondrial extracts but had no effect on the CAT activity in the cytosol. Treatment with Adrusen zinco did not influence the CAT activity in either mitochondrial or cytosol extracts of OXYS rat hepatocytes.

Interestingly, despite Adrusen zinco affected the protein carbonyl content, no remarkable changes in the CAT activity were found. Since the components of this supplement are lipid-soluble, the effect of Adrusen zinco may be mediated through inhibition

of oxidation of lipids and membrane proteins. Catalase mainly segregates to the aqueous phase, thus, the effect of Adrusen zinco may not be significant in this case.

Many polyphenols, including anthocyanins, have a marked antioxidant activity [24]. The antioxidant effect may reflect the ability of bilberry extracts to chelate transition metal ions involved in radical-forming processes such as the Fenton reaction [25]. Thus, an improvement in the antioxidant status of liver hepatocytes was expected as a result of consumption of Mirtilene forte.

In summary, this study demonstrates that both investigated antioxidants decrease the oxidative damage to proteins in the liver of 12-month old OXYS rats. At the same time they enhance the antioxidant defense system, exemplified by the catalase activity in mitochondria.

Discussion

ROS have been hypothesized to play an important role in the process of aging. A large body of experimental evidence supports a relationship between aging, genomic instability and damage to DNA or other components of living cells, including formation of 8-oxoguanine in DNA by hydroxyl radicals [26], age-related acceleration of peroxidation in a lens body [27], and an elevated SOD activity in the lenses of patients with cataracts [13, 14, 28]. The OXYS strain of rats may be very useful as a model to study the role of ROS in the process of aging, since this strain is characterized by ROS overproduction or by increased sensitivity to free radicals, which forms the basis of premature aging, short lifespan, low fertility, and increased frequency of cancers and chronic diseases (cataracts, cardiomyopathy, etc.) [13, 14, 29, 30].

In the present study, we have compared age-associated changes in the activities of antioxidant enzymes in cytosol and mitochondria of OXYS and Wistar rats. For OXYS rats, a decrease in the enzyme activity in 6-month-old rats compared to 3- and 12-months-old animals was observed both for CAT and SOD (Tables 2 and 3) in both cytosol and mitochondrial extracts, and the same age-dependent profile was found for the mitochondrial protein carbonyl content (Table 1). Such pattern for these enzymes is less pronounced in Wistar rats, but there is still a tendency towards a decrease in the activities in 6-month-old rats. At the same time, the activity of cytosol GPx did not decrease at 6 months of age. In addition, only GPx activity in the mitochondrial extracts increased (1.1–1.7-fold) in 6-month-old rats compared to 3- and 12-months-old rats.

Increased activity of mitochondrial CAT and GPx in young OXYS and Wistar rats may also be related to the processes of growth and the necessity for a stronger protection during this period.

Interestingly, 12-month-old OXYS rats demonstrated a significant increase in mitochondrial CAT activity compared with Wistar rats of the same age (Table 3). Thus, it is possible that the elements responsive to the oxidative stress could trigger an adaptive response that would increase the rate of damage removal, for example, through up-regulation of DNA repair enzymes and enhanced enzymatic protection of cells from oxidative damage.

Antioxidant enzymes are considered the primary defense that prevents biological macromolecules including proteins from oxidative damage. SODs rapidly convert $\cdot O^{2-}$ to less dangerous H_2O_2, which is further degraded by CAT and GPx to water. Thus, the elevated level of antioxidant enzymes during aging may protect important molecules such as DNA, proteins, and lipids against free radical-mediated damage.

Our findings concerning Wistar and OXYS rats agree with the reported data on the age-dependent changes in the levels of several antioxidant enzymes in Fisher rats [16]. For example, no significant difference in liver GPx between young and old rats was observed for Wistar and OXYS rats (Table 4) as well as for mitochondrial GPx of Fisher rats. The mitochondrial CAT activity of Wistar hepatocytes declined in an age-dependent manner (Table 3), also in agreement with previously published data for Fisher rats [16]. However, in contrast to Fisher rats, we have found a significant increase in the CAT activity in 12-months-old OXYS rats (Table 3). The MnSOD activity in the liver mitochondria of Fisher rats increases from 1-months-old to 6-months-old rats and then gradually declines with age [16]. In the liver mitochondria of Wistar rats, this activity was at its peak at 3 months, decreased 1.75-fold at 6 months and then increased again by a factor of 1.4 at 12 months. Nearly identical age-dependent changes in MnSOD were

observed in OXYS rats (Table 2). At the same time, age-dependent gradual increase in the Cu,ZnSOD activity in the cytosol of Wistar liver cells (Table 3) was similar to that for all cells of Fisher rats [16]. Thus, age-dependent changes in some antioxidant enzyme activities in cells of rats of different strains most probably can follow different patterns.

Interestingly, relative activities of SOD, CAT, and GPx in the cytosol and mitochondria of liver cells of Wistar and OXYS were generally higher in 12-months-old than in 6-months-old rats (Tables 2-4). While the level of protein carbonyl groups in the cytosol decreased in 12-months-old compared with 6-months-old rats, the damage to proteins was remarkably higher in the mitochondria of liver cells (Table 1). Thus, the observed increase in the levels of antioxidant enzymes in the mitochondria of rat liver cells was not sufficient to completely protect the mitochondrial components from oxidative damage. In addition, given a higher steady state level of ROS generation in OXYS than in Wistar rats [14], a statistically significant 1.24-fold increase in the rate of protein carbonyl group formation in the OXYS strain is not surprising. As can be seen from Table 2, mitochondria of OXYS were more proficient in the removal of ROS at all ages, especially at 3 and 12 months, compared to the Wistar mitochondria. Increased levels of some antioxidant enzymes in mitochondria compared to cytosol were also observed at all ages. These data are consistent with the existence of an adaptive response to ROS.

To investigate a correlation between oxidative stress and accumulation of DNA damage we have recently determined age-dependent levels of activities removing 8-oxoguanine, hypoxanthine and uracil from DNA in liver cells from OXYS rats in comparison with those of control Wistar rats [31]. A pronounced difference in the mitochondrial and nuclear 8-oxoguanine DNA glycosylase/AP lyase activities were revealed in both cases. Our results demonstrated an induction of 8-oxoG-, uracil- and hypoxanthine-specific repair pathway with age in both types of rats. The 8-oxoguanine DNA glycosylase/AP lyase activity in the mitochondrial extracts of old OXYS rats was remarkably higher than in old Wistar rats.

Taken together, the more pronounced increase in the 8-oxoguanine DNA glycosylase/AP lyase and CAT activities in OXYS compared to Wistar rats suggest the accelerated induction of these protective activities during aging in OXYS rats. However, this is insufficient to prevent faster oxidative damage of proteins and DNA of OXYS rats, and these rats age faster than Wistar rats.

Conclusions

A faster age-dependent increase in the level of protein carbonyl groups was found in OXYS as compared with Wistar rats. Complicated enzyme-specific patterns of age-dependent changes in the activities of antioxidant enzymes were observed.

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