Vol. 196, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1993 Pages 7-11

BIOCHEMICAL CORRELATES OF LONGEVITY IN TWO CLOSELY RELATED RODENT SPECIES

R.S. Sohal*, Hung-Hai Ku and Sanjiv Agarwal

Department of Biological Sciences, Southern Methodist University, Dallas, Texas 75275

Received August 23, 1993

SUMMARY - The objective of this study was to explore the basis of variations in the life span and metabolic potential, i.e., total amount of energy consumed during life, between different species, in context of the free radical hypothesis of aging. A comparison was made between the house mouse (*Mus musculus*) and the white-footed mouse (*Peromyscus leucopus*): the latter has >2-fold greater life span and metabolic potential than the former. Longer life span and higher metabolic potential of *Peromyscus* were associated with low rates of mitochondrial $O_2^{\cdot-}$ and H_2O_2 generation, higher activities of catalase and glutathione peroxidase and low levels of protein oxidative damage as well as low susceptibility to oxidative damage in response to experimental oxidative stress. Results support the role of oxidative stress in aging.

The nature of the mechanisms underlying the aging process is presently not well understood. Several different investigative strategies and hypotheses have been proposed to identify the constitutional characteristics of organisms, which govern their rate of aging. One strategy, which has also been employed here, involves comparison of species that are closely-related phylogenetically, but have different species-specific maximum life span potential (MLSP). In the present study, a comparison was made between two rodents namely, the house mouse (*Mus musculus*) with an MLSP of 3.5 years, and the white-footed mouse (*Peromyscus leucopus*) with an MLSP of 8 years (1).

A currently popular hypothesis postulates the involvement of oxygen free radicals and hydroperoxides, collectively termed as 'reactive oxygen species' (ROS), and constantly produced in cells, in the aging process. It is believed that a small proportion of ROS escape elimination by antioxidant defenses, consequently inflicting oxidative damage to cellular molecules. Some of the oxidative damage is thought to be irreparable, thereby accumulating with age, and contributing to the age-associated loss in homeostatic efficiency (2, 3). In short, this hypothesis predicts that the rate of aging is dependent on the level of oxidative stress, i.e., the balance between pro-oxidants and antioxidants, and the consequent oxidative damage. The purpose of the present study is to test this prediction.

ROS are initially produced by the univalent reduction of dioxygen, generating $O_2^{\cdot-}$ and H_2O_2. The latter, if not eliminated, can produce the hydroxyl radical, which is widely believed

* Corresponding author.

0006-291X/93 $4.00

to be the main cause of oxidative molecular damage (4), including carbonyl modifications in cellular proteins (5). The primary antioxidant defenses are provided by superoxide dismutase (SOD), which converts $O_2^{\cdot-}$ to H_2O_2, and catalase and glutathione peroxidase, which together eliminate H_2O_2 (5).

Present study reports a comparison of rates of mitochondrial $O_2^{\cdot-}$ and H_2O_2 generation, activities of SOD, catalase and glutathione peroxidase, protein oxidative damage as well as the susceptibility to undergo oxidative damage in the brain and heart of *Mus* and *Peromyscus*.

MATERIALS AND METHODS

Heart and brain were obtained from about 3.5-month old male *Mus musculus* (Rockland Wistar) and *Peromyscus leucopus*. The average weight of *Mus* was 40 gm and *Peromyscus* about 19 gm. The biochemical procedures used in this study have been described in detail elsewhere and are thus only referred to here. Mitochondria were isolated by the procedure of Arcos *et al.* (6) in the heart and of Ozawa *et al.* (7) in the brain, as described previously (8). $O_2^{\cdot-}$ production was measured in submitochondrial particles as SOD-inhibitable reduction of acetylated cytochrome *c* using succinate as a substrate and antimycin A as a respiratory inhibitor (8). H_2O_2 release by intact mitochondria was measured fluorometrically by monitoring the oxidation of *p*-phenylhydroxyacetic acid, catalyzed by horseradish peroxidase (8). Succinate was used as a substrate.

Activities of antioxidant enzymes were measured in tissue homogenates. SOD activity was measured by the "direct" method of Misra and Fridovich (9), catalase activity according to Luck (10) and glutathione peroxidase activity by the method of Paglia and Valentine (11) as described previously (8).

X-irradiation was conducted in clear plastic containers using Philips RT305 X-ray generator operating at 300 kV, 10 mA and a dose rate of 200 r/min. Protein carbonyl content was measured by the method of Levine *et al.* (12) using 2,4 dinitrophenylhydrazine, as described previously (13).

RESULTS

Specific metabolic rate (SMR) of the animals was calculated using Kleiber's equation (14):

$$SMR\left(\text{cal}\cdot\text{gm}^{-1}\cdot\text{day}^{-1}\right) = 393(\text{gm body weight})^{-0.25}.$$

Accordingly, the metabolic rate of *Mus* is 156- and of *Peromyscus* 188 $\text{cal}\cdot\text{gm}^{-1}\cdot\text{day}^{-1}$. The metabolic potential, i.e. the total amount of energy consumed during life, for *Mus* would be 200 $\text{kcal}\cdot\text{gm}^{-1}$ and for *Peromyscus* 550.

A comparison of the rates of oxidant production, antioxidant defenses and protein carbonyl content between *Mus* and *Peromyscus* was made in the heart (Table 1), and the brain (Table 2). Rate of $O_2^{\cdot-}$ was measured in submitochondrial particles using succinate as a substrate and antimycin A as a respiratory inhibitor, which implies that ubisemiquinone/cytochrome *b* region is the putative site of $O_2^{\cdot-}$ generation, measured in this study. The rate of $O_2^{\cdot-}$ generation was notably higher in *Mus* than *Peromyscus*, being 74% greater in the heart ($p = 0.0003$) and 48% in the brain ($p = 0.0009$). The rate of H_2O_2 released by mitochondria was also greater in *Mus* than *Peromyscus*, the difference was 5.5-fold in the heart ($p = 0.0001$, Table 1) and 3-fold in the brain ($p = 0.0001$, Table 2).

Table 1. Rates of pro-oxidant generation, antioxidant defenses and protein oxidative damage in the heart of *Mus* and *Peromyscus*

Parameter	*Mus*	*Peromyscus*
Rates of Pro-oxidant Generation		
1. Mitochondrial $O_2^{\cdot-}$ production (μmol · min $^{-1}$· mg protein $^{-1}$)	4.12 ± 0.22	2.37 ± 0.06
2. Mitochondrial H_2O_2 release (nmol · min^{-1}· mg protein $^{-1}$)	2.32 ± 0.08	0.42 ±0.02
Antioxidant Levels		
3. Superoxide dismutase activity (units · mg protein $^{-1}$)	41.3 ± 0.60	46.9 ± 0.90
4. Catalase activity (units · mg protein $^{-1}$)	3.45 ± 0.05	7.95 ± 0.12
5. Glutathione peroxidase activity (units · μg protein $^{-1}$)	20.1 ± 0.77	47.0 ± 0.94
Oxidative Damage		
6. Protein carbonyl content (nmol · mg protein $^{-1}$)	1.21 ± 0.02	0.99 ± 0.04

Note: Values are average ± SEM of 3-8 measurements.

SOD activity was only marginally higher in *Peromyscus* than *Mus*, however, catalase and glutathione peroxidase activities were sharply higher in the former than the latter. The magnitude of the difference in catalase activity was 2.3-fold in the heart (p = 0.0001, Table 1) and 1.5-fold in the brain (p = 0.0001, Table 2). Glutathione peroxidase activity in *Peromyscus* was 2.3-fold higher in the heart (p = 0.0001) and 3-fold greater in the brain (p = 0.0001), as compared to *Mus*.

To compare the relative susceptibility of the two species to experimental oxidative stress, tissue homogenates were exposed to different levels of X-irradiation. As shown in Table 2 and Fig. 1, the protein carbonyl concentration in the brain of unirradiated *Mus* was higher than in the unirradiated *Peromyscus* (p = 0.0001). As indicated by the slope of the best-fit lines, the rate of

Table 2. Rates of pro-oxidant generation, antioxidant defenses and protein oxidative damage in the brain of *Mus* and *Peromyscus*

Parameter	*Mus*	*Peromyscus*
Rates of Pro-oxidant Generation		
1. Mitochondrial $O_2^{\cdot-}$ production (μmol · min $^{-1}$· mg protein $^{-1}$)	0.68 ± 0.02	0.46 ± 0.01
2. Mitochondrial H_2O_2 release (nmol · min^{-1}· mg protein $^{-1}$)	0.64 ± 0.04	0.19 ± 0.02
Antioxidant Defenses		
3. Superoxide dismutase activity (units · mg protein $^{-1}$)	32.4 ± 0.61	35.2 ± 1.00
4. Catalase activity (units · mg protein $^{-1}$)	0.56 ± 0.01	0.84 ± 0.01
5. Glutathione peroxidase activity (units · μg protein $^{-1}$)	8.35 ± 0.53	25.6 ± 0.53
Oxidative Damage		
6. Protein carbonyl content (nmol · mg protein $^{-1}$)	1.97 ± 0.04	1.10 ± 0.03

Note: Values are average ± SEM of 3-8 measurements.

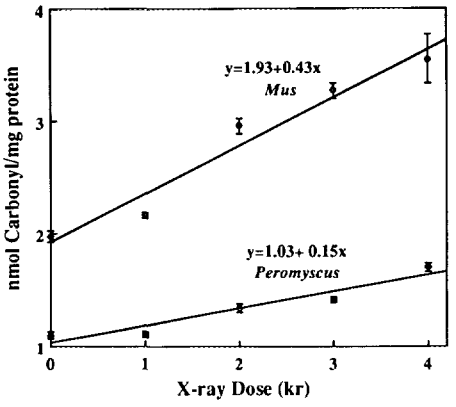

Fig. 1. **Effect of various dosages of X-irradiation on the concentration of protein carbonyls in the homogenates of the brain of Mus and Peromyscus.** Tissues were homogenized in 5 mM phosphate buffer (10% W/V), pH 7.4, 0.1% Triton-X and protease inhibitors, aprotinin, leupeptin and pepstatin.

increase in carbonyl content of the brain homogenates in response to different doses of X-irradiation was almost 3-times steeper in *Mus* than *Peromyscus*.

Homogenates of the heart were exposed to a single dose of 4 kr of X-irradiation. The concentration of protein carbonyls in the unirradiated controls was 22% higher in *Mus* as compared to *Peromyscus* (p = 0.003, Table 1), however, the difference increased to 63% after 4 kr irradiation (2.3 nmole/mg protein in *Mus* vs. 1.43 in *Peromyscus*).

DISCUSSION

Results of this and our previous studies suggest the existence of a characteristic pattern associated with MLSP and metabolic potential in different vertebrate species. MLSP is inversely related to the rates of mitochondrial oxidant generation. For example, rates of mitochondrial $O_2^{\cdot-}$ and H_2O_2 production in the liver (15, 16), heart and kidney (17) have been found to be inversely correlated to MLSP of seven different mammalian species. In contrast, the role of antioxidant defenses as determinants of MLSP appears to be relatively more ambiguous and complex. It seems that antioxidant defenses are positively associated with metabolic potential, but not necessarily with MLSP. For example, a comparison of SOD, catalase, glutathione peroxidase and glutathione in tissues of six different mammalian species namely, mouse, rat, guinea pig, rabbit, pig and cow, which have a relatively similar metabolic potential of about 150-250 kcal/gm body weight, respectively, indicated the lack of a consistent association between antioxidant defenses and MLSP of these species (18). In contrast, a comparison of rat and pigeon, which have metabolic potentials of about 150- and >800-kcal/gm body weight, indicated that the antioxidant defenses in the pigeon were notably higher than in the rat (19). Results of the present study clearly demonstrate that antioxidant defenses, especially the efficiency of H2O2 elimination, are notably higher in *Peromyscus* than *Mus*. This inference is further strengthened by the present finding that the tissues of *Peromyscus* are less susceptible to experimentally-induced oxidative damage, as indicated by protein carbonyl content, than of *Mus*.

Considered together, it seems that relatively low rates of oxidant generation are associated with longer MLSP whereas a combination of low rates of oxidant generation and high levels of antioxidant defenses characterize elevated metabolic potential.

ACKNOWLEDGMENTS

This research was supported by grant R01AG7657 from National Institutes of Health, National Institute on Aging. We are indebted to Mr. Richard C. Kebart and Dr. G. Mues, Mary C. Crowley Medical Research Program, Baylor Research Institute, Dallas for the use of the X-ray source.

REFERENCES

1. Sacher, G. A. and Hart, R. W. (1978) Birth Defects 14, 71-96.
2. Harman, D. (1981) Proc. Natl. Acad. Sci. USA 78, 7124-7128.
3. Sohal, R. S. and Allen, R. G. (1986) Adv. Free Rad. Biol. Med. 2, 117-160.
4. Fridovich, I. (1978) Science 201, 875-880.
5. Stadtman, E. R. and Oliver, C. N. (1991) J. Biol. Chem. 266, 2005-2008.
6. Arcos, J. C., Sohal, R. S., Sun, S. C., Argus, M. F., and Burch, G. E. (1968) Exp. Mol. Path. 8, 49-65.
7. Ozawa, K., Seta, K., Takeda, H., Ando, K., Handa, H., and Araki, C. (1966) J. Biol. Chem. 59, 501-510.
8. Sohal, R. S., Arnold, L. A., and Sohal, B. H. (1990) Free Rad. Biol. Med. 10, 495-500.
9. Misra, H. P. and Fridovich, I. (1977) Anal. Biochem. 79, 553-560.
10. Luck, H. (1965) In Methods of Enzymatic Analysis (H. Bergmeyer, Ed.), pp. 885-894. Academic Press, New York.
11. Paglia, D. E. and Valentine, W. N. (1967) J. Lab. Clin. Med. 70, 158-169.
12. Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S., and Stadtman, E. (1990) Meth. Enzymol. 186, 464-478.
13. Sohal, R. S., Agarwal, S., Dubey, A., and Orr, W. C. (1993) Proc. Natl. Acad. Sci. USA 90, 7255-7259.
14. Kleiber, M. (1947) Physiol. Rev. 27, 511-541.
15. Sohal, R. S., Svensson, I., Sohal, B. H., and Brunk, U. T. (1989) Mech. Ageing Dev. 49, 129-135.
16. Sohal, R. S., Svensson, I., and Brunk, U. T. (1990) Mech. Ageing Dev. 53, 209-215.
17. Ku, H.-H., Brunk, U. T., and Sohal, R. S. (in press) Free Rad. Biol. Med.
18. Sohal, R. S., Sohal, B. H., and Brunk, U. T. (1990) Mech. Ageing Dev. 53, 217-227.
19. Ku, H.-H. and Sohal, R. S. (in press) Mech. Ageing Dev.