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LONG-LIVED AMES DWARF MICE: OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA IN HEART AND BRAIN

Alberto Sanz*, Andrzej Bartke$ and Gustavo Barja*
$ Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, Illinois 62901, USA
* Department of Animal Biology-II (Animal Physiology), Faculty of Biology, Complutense University, Madrid 28040, Spain.

ABSTRACT

The single gene mutation of Ames dwarf mice increases their maximum longevity by around 40% but the mechanism(s) responsible for this effect remain to be identified. This animal model thus offers a unique possibility of testing the mitochondrial theory of aging. In this investigation, oxidative damage to mitochondrial DNA (mtDNA) was measured for the first time in dwarf and wild type mice of both sexes. In the brain, 8-oxo,7,8-dihydro-2'-deoxyguanosine (8-oxodG) in mtDNA was significantly lower in dwarfs than in their controls both in males (by 32%) and in females (by 36%). The heart of male dwarfs also showed significantly lower mtDNA 8-oxodG levels (30% decrease) than the heart of male wild type mice, whereas no differences were found in the heart of females. The results, taken together, indicate that the single gene mutation of Ames dwarfs lowers oxidative damage to mtDNA especially in the brain, an organ of utmost relevance for aging. Together with the previous evidence for relatively lower level of oxidative damage to mtDNA in both long-lived and caloric restricted animals, these findings suggest that lowering of oxidative damage to mtDNA is a common mechanism of life extension in these three different mammalian models.

INTRODUCTION

Ames (1) and Snell (2) dwarf mutant mice have around 40% longer mean and maximum life span than normal controls, and constitute the first mammalian models of decelerated aging due to single gene mutations. It is thus extremely interesting to unravel the mechanisms responsible for the slow aging rate of these mice. The mutations lead in both dwarfs to deficient secretion of growth hormone (GH), prolactin and thyroid stimulating hormone (3). Previous studies suggest that GH deficiency is the most critical endocrine change functionally linked to the slowly aging phenotype. However, there is no information concerning the intracellular mechanisms by which lack of GH leads to decelerated aging.

Among theories of aging, the mitochondrial free radical theory is supported by various lines of scientific evidence. It is known that mitochondrial DNA (mtDNA), which is situated near the main generator of reactive oxygen species (the inner mitochondrial membrane), accumulates somatic mutations during aging in post-mitotic tissues and is more heavily damaged than nuclear DNA (4-6). Recent studies indicate that the heart and brain mtDNA of long-lived mammals have lower levels of oxidative damage (measured as 8-oxodG; 8-oxo,7,8-dihydro-2'-deoxyguanosine) than those of short-lived mammals (6). Furthermore, caloric restriction, the only experimental intervention known to decrease the rate of aging, also decreases 8-oxodG in rat heart mtDNA without influencing the damage of nuclear DNA (7). Interestingly, it has been observed that dwarf mutant mice share many characteristics with caloric restricted rodents (8), although their delayed aging is not due to restriction of calories (9). It is possible that the intracellular mechanisms of delayed aging in the dwarfs involve a decrease in mitochondrial free radical attack on mtDNA. However, the degree of oxidative damage to the mtDNA of these mutant dwarf mice has never been studied. Thus, in this study we have measured the steady-state levels of 8-oxodG in the mtDNA of heart and brain (two post-mitotic vital organs) of male and female Ames dwarf mice and wild type corresponding controls.

RESULTS

Oxidative damage to mtDNA in the brain of male and female Ames dwarf and wild type mice is shown in Fig. 1. A two-way analysis of variance indicated that 8-oxodG is significantly lower in the brain of dwarfs than in controls in both sexes, whereas there was no effect of sex or interaction between sex and mutation on brain 8-oxodG levels (Fig. 1). The 8-oxodG concentration was 32% lower in male dwarfs than in male controls and 36% lower in female dwarfs than in female controls.

In the heart, no effects of the Ames mutation, sex, or interaction were found by two-way analysis of variance. However, separate analysis by Student's t tests showed that heart 8-oxodG was significantly lower (by 30%) in male dwarfs than in male controls, whereas no differences were found in females (Fig. 2). 8-oxodG was also significantly lower in female than in male wild type mice (Student's test).

When males and females are considered together, Ames dwarf mice showed significantly lower 8-oxodG than wild type controls in heart and brain mtDNA.

Mailing Address:
Prof. Dr. G. Barja.
Departamento de Biología Animal-II (Fisiología Animal)
Facultad de Biología, Universidad Complutense, Madrid 28040, Spain.
Tel. 34-91-3944919. FAX: 34-91-3944935.

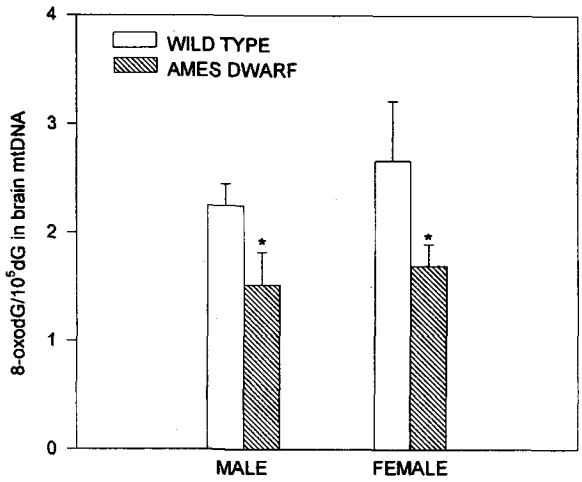

Figure 1: Oxidative damage to brain mitochondrial DNA in Ames dwarf and wild type male and female mice. *: significant difference between Ames dwarf and control mice of the same sex. Values are means ± SEM from 6-8 different samples.

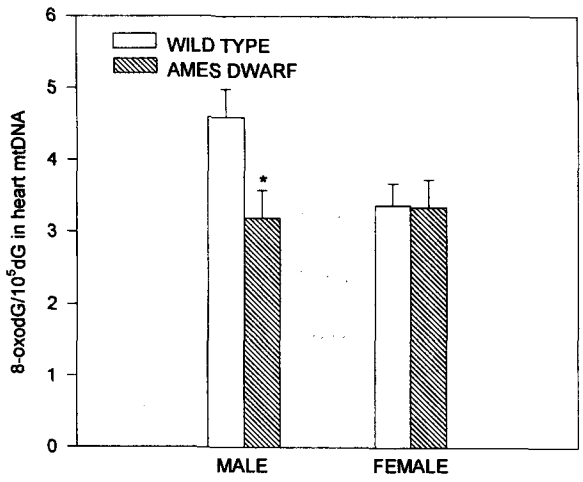

Figure 2: Oxidative damage to heart mitochondrial DNA in Ames dwarf and wild type male and female mice. *: significant difference between Ames dwarf and control mice of the same sex. Values are means ± SEM from 4-7 different samples.

DISCUSSION

Although many studies mainly concerning blood hormones have been performed in dwarf mutant mice (3), the intracellular mechanisms of delayed aging operating in these animals are unknown. This is the first study reporting levels of oxidative damage to mtDNA in long-lived dwarf mice. Previous studies concerning oxidative stress are mainly limited to tissue antioxidant levels (10,11) with some investigations reporting inorganic and lipid peroxides (12) and oxidative damage to proteins and nuclear DNA (13). We show here that 8-oxodG in mtDNA is lower in the brain of dwarfs of both sexes and in the heart of male dwarf mice than in the corresponding organs of wild type mice. Only the heart of female dwarfs showed similar 8-oxodG to controls. The more coherent pattern of the brain fits well with the main role of this organ in aging, since it is the main regulator and coordinator of all other organs in the body. On the other hand, although the reason for the lack of differences in female heart is unknown, it can be related to the lower levels of 8-oxodG already observed in female than in the male wild type controls. Longevity is generally longer in female than in male mammals including humans, and lower levels of 8-oxodG have been accordingly observed in women than in men (14-16). These differences in longevity seem to be due to the cardioprotective effect of estrogens in the females (17). In relation to this, recent experimental studies of ovariectomy plus estrogen replacement have demonstrated that the lower 8-oxodG levels in the mtDNA of female versus male rodents are due to estrogens (18). Ames dwarfs are hypogonadal. Thus, the protective effect of estrogens are probably reduced in dwarfs in comparison to their normal siblings. Convergence into a common final intracellular mechanism could also be involved in lowering of 8-oxodG in the heart of females and in dwarfism, which would explain why the effects of sex and the mutation are not additive in this particular organ. Other explanations of the sex differences observed in the heart mtDNA in the present investigation are also possible. First, Ames dwarf females live significantly longer than Ames dwarf males. In the present investigation 8-oxodG was measured at a single time point. Since Ames dwarfism seems to delay aging, changes in the female heart may show up at a later age than in male dwarf mice. Second, similarly to caloric restricted rodents, lower levels of insulin have been found in dwarfs than in wild type controls in males (19, 20), whereas this was not the case in non-fasted females (19).

According to the present version of the mitochondrial theory of aging, mitochondrial generation of oxygen radicals damages mtDNA, leading to the life-long accumulation of mtDNA mutations, progressive mitochondrial dysfunction, and aging. In agreement with this theory, both long-lived animals and caloric restricted animals show decreased levels of mitochondrial oxygen radical generation and 8-oxodG in mtDNA in their heart and brain (6,7,21). The results obtained in this report show that 8-oxodG is also diminished in the mitochondria of long-lived Ames dwarf mice. This decrease suggests that mitochondrial free radical generation is also lower in dwarfs than in wild type mice, a change possibly involved in the superior longevity of the dwarfs. Previous studies have shown that long-lived dwarf mice share many characteristics with caloric restricted animals (8) and that, among endocrine changes, life extension in dwarfs is mainly related to depletion of blood GH and IGF-1 (3). On the other hand, the main hormone decreasing in the blood of caloric restricted rodents is insulin, and IGF-1 is also decreased during caloric restriction (22). Those two endocrine pathways (GH and insulin) can have intracellular effects through binding to common receptors or overlapping mechanisms. Thus, it is possible that the low blood GH/IGF-1 or insulin levels of dwarf mutants or caloric restricted animals signal mitochondria, possibly via IGF-1 receptors, to decrease their levels of free radical generation and thus mtDNA oxidative damage. It is striking that recent information suggests that insulin/IGF-1-like signaling seems also to be involved in life extension in invertebrates including *C. elegans* and *D. melanogaster* (23), models in which there is evidence

that decreases in oxidative stress are involved in the determination of the long-lived phenotypes. Thus, present information indicates that a small number of fundamental signaling mechanisms speed up or slow down aging in different species, mutants or individuals subjected to different availability of energy sources. In mammals these mechanisms appear to lower oxidative stress in mitochondria and therefore decrease oxidative damage to mitochondrial DNA. In summary, available results indicate that a low level of oxidative damage in the mitochondrial DNA is a common trait in the three different models of mammalian life extension so far investigated: long-lived animals, caloric restriction, and the Ames dwarf single gene mutation.

EXPERIMENTAL PROCEDURES

Ames dwarf (Prop1df) mutant mice were produced in a Southern Illinois University closed breeding colony in a fully AAALAC-accredited Vivarium by mating heterozygous carriers of this mutation or by mating homozygous dwarf males with heterozygous dwarf females. Normal siblings of dwarf mice served as controls. After weaning, the animals were separated by sex and, for males, also by genotype and housed in micro-isolator cages, 4-5 animals per cage in a room with controlled illumination (12 h light:12 h darkness) and temperature (22±2°C) with constant access to food (5008f Mouse Diet; PMI, Brentwood, MO) and tap water. Monitoring of sentinel animals from the same room indicated that the animals were sero-negative for all common mouse pathogens.

At the age of 4.5-7 months, the animals were killed by decapitation, the organs were removed, rapidly frozen and stored at -70°C and shipped to Complutense University (Spain), where they were kept at -70°C until used for 8-oxodG analysis. After initial homogenization of the organs in a buffer containing 5mM EDTA, mtDNA was isolated by the method of Latorre et al. (24) adapted to mammals (6,7). mtDNA digestion to deoxynucleosides and 8-oxodG and dG HPLC analyses were performed as described (25). The amount of mtDNA analyzed was 28.6-34.8 μg in heart and 64.8-79.9 μg in brain. The limit of detection was around 10 μg of DNA. All aqueous solutions used for DNA isolation, digestion and chromatographic separation were prepared in HPLC-grade water (Fisher Chemicals, Loughborough, UK). Data were statistically analyzed by two-way analyses of variance and Student's t tests, selecting $p<0.05$ as the minimum level of significance.

ABBREVIATIONS

8-oxodG: 8-oxo,7,8-dihydro-2'-deoxyguanosine
dG: deoxyguanosine
mtDNA: mitochondrial DNA
HPLC: high performance liquid chromatography

REFERENCES

1. Brown-Borg, H.M., Borg, K.E., Meliska, C.J., and Bartke, A.: Dwarf mice and the aging process. Nature, 384: 33, 1996.
2. Flurkey, K., Papaconstantinou, J., Miller, R.A., and Harrison, D.E.: Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. PNAS, 98: 6736-6741, 2001.
3. Bartke, A., Brown-Borg, H., Mattison, J., Kinney, B., Hauck, S., and Wright, C.: Prolonged longevity of hypopititary dwarf mice. Exp. Gerontol., 36: 21-28, 2001.
4. Napiwotzki, J., Reith, A., Becker, A., Leist, S., and Kadenbach, B.: Quantitative analysis of mutations of mitochondrial DNA during human aging, in Understanding the Process of Aging, edited by Cadenas, E. and Packer, L. New York, Marcel Dekker, 1999, pp. 251-264.
5. Richter, Ch., Park, J.W., and Ames B.N.: Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc. Natl. Acad. Sci. USA, 85: 6465-6467, 1988.
6. Barja, G., and Herrero A.: Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. FASEB Journal, 14: 312-318, 2000.
7. Gredilla, R., Sanz, A., Lopez-Torres, M., and Barja, G.: Caloric restriction decreases mitochondrial free radical generation at Complex I and lowers oxidative damage to mitochondrial DNA in the rat heart. FASEB Journal, 15: 1589-1591, 2001.
8. Mattison, J.A., Wright, C., Bronson, R.T., Roth, G.S., Ingram, D.T. and Bartke, A.: Studies of aging in Ames dwarf mice: Effects of caloric restricition. J. Amer. Aging Assoc., 23: 9-16, 2000.
9. Bartke, A., Wright, J.C., Mattison, J.A., Ingram, D.K., Miller, R.A., and Roth, G.: Longevity: extending the lifespan of long-lived mice. Nature, 414: 412, 2001.
10. Brown-Borg, H.M., Bode, A.M., and Bartke, A.: Antioxidative mechanisms and plasma growth hormone levels. Endocrine, 11: 41-48, 1999.
11. Hauck, S.J., and Bartke, A.: Effects of growth hormone on hypotalamic catalase and Cu/Zn superoxide dismutase. Free Rad. Biol. Med., 28: 970-978, 2000.
12. Carlson, J.C., Bharadwaj, R. and Bartke, A.: Oxidative stress in hypopituitary dwarf mice and in transgenic mice overexpressing human and bovine GH. Age-Media, 22:181-186, 1999.
13. Brown-Borg, H., Johnson, W.T., Rakoczky, S. and Romanick, M.: Mitochondrial oxidant generation and oxidative damage in Ames dwarf and GH transgenic mice. J. Amer. Aging Assoc., 24:85-96, 2001.
14. Collins, A.R., Gedik, C.M., Olmedilla, B., Southon, S., and Bellizzi, M.: Oxidative damage measured in human lymphocytes: large differences between sexes and between countries, and correlations with heart disease mortality rates. FASEB J., 12: 1397-1400, 1998.
15. Chen, L., Bowen, P.E., Berzy, D., Aryee, F., Sapuntzakis, M., and Riley, R.E.: Diet modification affects DNA oxidative damage in healthy humans. Free Rad. Biol. Med., 26: 695-703, 1999.

16. Loft, S., Ewertz, M., Tjonneland, A., Overvad, K., and Poulsen, H.E.: Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans-influence of smoking, gender and body mass index. Carcinogenesis, 13: 2241-2247, 1992.
17. Asdell, S.A., Doornenbal, H., Joshi, S.R., and Sperling, G.A.: The effects of sex steroid hormones upon longevity in rats. J. Reprod. Fert., 14: 113-120, 1967.
18. Sastre, J., Borras, C., García-Sala, D., Lloret, A., Pallardo, F.V. and Viña, J.: Mitochondrial damage in aging and apoptosis. Ann. New York Acad. Sci., 959: 448-451, 2002.
19. Borg, K.E., Brown-Borg, H.M., and Bartke, A.: Assessment of the primary adrenal cortical and pancreatic hormone basal levels in relation to plasma glucose and age in the unstresses Ames dwarf mouse. Proc. Soc. Exper. Biol. Med., 210: 126-133, 1995.
20. Dominici, F.P., Hauck, S., Argentino, D.P., Bartke, A. and Turyn, D.: Increased insulin sensitivity and upregulation of insulin receptor, insulin receptor substrate (IRS)-1 and IRS-2 in liver of Ames dwarf mice. J. Endocrinol., 173: 81-94, 2002.
21. Barja, G.: Mitochondrial Free Radical Generation: Sites of production in states 4 and 3, specificity and relationship with aging rate. J. Bioenerg. Biomembr., 31: 347-366, 1999.
22. Breese, C.R., Ingram, R.L., and Sonntag, W.E.: Influence of age and long-term dietary restriction on plasma insulin-like growth factor (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. J. Gerontol. Biol. Sci., 46: B180-B1877, 1991.
23. Kenyon, C.: A conserved regulatory system for aging. Cell, 105: 165-168, 2001.
24. Latorre, A., Moya, A., and Ayala, A.: Evolution of mitochondrial DNA in Drosophila suboscura. Proc. Natl. Acad. Sci. USA., 83: 8649-8653, 1986.
25. Loft, S., and Poulsen, H.E.: Markers of oxidative damage to DNA: antioxidants and molecular damage. Methods in Enzymology, 300: 166-184, 1999.