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Regular exercise reduces 8-oxodG in the nuclear and mitochondrial DNA and modulates the DNA repair activity in the liver of old rats

Hideko Nakamoto [a], Takao Kaneko [b], Shoichi Tahara [b], Eri Hayashi [a], Hisashi Naito [c], Zsolt Radak [d], Sataro Goto [a,*]

[a] *Department of Biochemistry, Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan*
[b] *Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*
[c] *Department of Sports Physiology, School of Sports Sciences, Juntendo University, Japan*
[d] *Laboratory of Exercise Physiology, School of Sport Science, Semmelweis University, Budapest, Hungary*

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Abstract

Exercise is often said to increase the generation of reactive oxygen species that are potentially harmful. On the other hand, regular exercise has various health benefits even late in life. The specific aim of this study was to explore effects of regular exercise on oxidative status of DNA in aged animals. We report that 2 months of regular treadmill running of aged rats (21 month old) significantly reduced 8-oxodG content to the level of young adult animals (11 month old) in both nuclear and mitochondrial DNA of the liver. The mitochondrial DNA showed 10-fold higher content of the oxidative lesion than the nuclear DNA. The levels in old animals were 2- and 1.5-fold higher than that in young adults for the nucleus and mitochondria, respectively. The activity of the repair enzyme OGG1 was upregulated significantly in the nucleus but not in mitochondria by the exercise. To our knowledge, this is the first report demonstrating that regular exercise can reduce significantly oxidative damage to both the nuclear and mitochondrial DNA. We suggest that the apparent beneficial outcomes in reducing the DNA damage by regular exercise can be interpreted in terms of hormetic effect by moderate oxidative stress and potential adaptation to stronger stresses.

Keywords: Aging; Liver; Regular exercise; DNA oxidation; 8-oxodG; DNA repair; OGG1; Nucleus; Mitochondria

1. Introduction

Reactive oxygen species (ROS) unavoidably generated in normal energy metabolism in mitochondria as well as in physiological enzymatic processes have been implicated in aging and age-related diseases since the seminal paper of Denham Harman (Harman, 1956). Nucleic acids, proteins and lipids are subjected to modifications by ROS in aerobic organisms, potentially leading to functional impairment of these vital molecules and thus decreasing cellular activities with advancing age if not repaired (Beckman and Ames, 1998; Martin et al., 1996). To cope with such detrimental consequences cells are equipped with multiple antioxidants and antioxidative enzymes as the primary and secondary defense, respectively (Finkel and Holbrook, 2000). In addition, oxidative damage, if it occurred, can be repaired or the damaged molecules replaced by intact ones as the tertiary defense mechanism (Davies, 1986). Nevertheless, the damage can accumulate with time due to incomplete defenses, leading to increased vulnerability and to eventual death of an organism. Investigators have, therefore, tested numerous natural and synthetic antioxidative chemicals in an attempt to retard aging and attenuate age-related oxidative diseases, but with limited success except for improving some pathological conditions. For example, vitamins C and E,

* Corresponding author. Tel./fax: +81 47 472 1531.
E-mail address: goto@phar.toho-u.ac.jp (S. Goto).

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well-appreciated antioxidants, have failed to reduce oxidative stress and other detrimental consequences in apparently healthy individuals (Herbert et al., 2006; Miller et al., 2005; Vivekananthan et al., 2003) while deficiency of these vitamins obviously induces oxidative stress and other disorders. This is, however, not unexpected in view of the fact that cellular redox systems are in a delicate balance that may be disturbed by an uncontrolled intake of such chemicals (Bailey et al., 2006; Galati et al., 2006). On the other hand, antioxidant enzymes may be induced or activated in reducing oxidative damage by other means such as dietary or caloric restriction (Nagai et al., 2000; Sanz et al., 2005) and exercise (Judge et al., 2005; Radák et al., 1999, 2001, 2004). We have reported that regular swimming exercise can attenuate oxidative damage to proteins and/or DNA in the skeletal muscle (Radák et al., 1999) and brain (Radák et al., 2001) in rats. Oxidative stress was reduced in the liver as well by regular treadmill exercise training in rats (Radák et al., 2004). These findings illustrate beneficial mechanisms of the exercise, if intensity were appropriate, in reducing oxidative stress rather than increasing it, contrary to what is generally believed. We have proposed that a moderate level of regular exercise can be a form of hormesis in that while excessive exercise to an unprepared body is likely harmful due to massive generation of ROS that exceeds the antioxidative capacity of cells, moderate exercise can be beneficial inducing an adaptive response to cope with higher oxidative and possibly other stresses that may be encountered later (Radák et al., 2005). The notion of hormesis that was described originally in radiobiology has now been extended to a variety of medical and biological domains including toxicology and gerontology (Calabrese, 2004, 2006; Rattan, 2004, 2005).

Oxidative modifications of DNA bases in the nuclei and mitochondria have been suggested as a mechanism of biological aging (Ames et al., 1993; Hamilton et al., 2001; Harman, 1972) and age-related diseases (Cooke et al., 2003; Lombard et al., 2005), particularly cancer (Jackson and Loeb, 2001; Loft and Poulsen, 1996). We have been interested in beneficial the effects of regular exercise, focusing on possible attenuation of oxidative stress in rodent (Goto et al., 2004) as well as in human (Radák et al., 2003). The specific aim of the present study was to examine whether or not exercise of regular treadmill running can reduce oxidative DNA damage in nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) of old rat livers.

2. Materials and methods

2.1. Animals and exercise protocol

Male specific pathogen free (SPF) F344/Du rats were purchased from the Tokyo Metropolitan Institute of Gerontology at the age of 11 and 21 month old. They were kept under clean conventional conditions in our animal facility of Faculty of Pharmaceutical Sciences, Toho University (Radák et al., 2004) until they were killed for the excision of tissues 1 week, for the younger adult animals, or 8 weeks, for the older animals, after the regular exercise period. Sixteen older animals were familiarized with walking on a motor-driven treadmill for 5 days at 5–7 m/min, once a day for 10–20 min. Five adult animals were similarly handled for 5 days. After this acclimation period, 8 old animals each were randomly assigned to either a sedentary control or an exercise trained group. The animals of training group ran 30–40 min per day at 8–10 m/min with an 8% slope for the first 2 weeks and then for gradually increasing times at higher speed up to 90 min per day at 14–15 m/min in the last 3 weeks. During the last 3 weeks the slope was deceased to 5% because the motivation of running appeared to be reduced. A soft brush but not electric shock was sometimes used manually to stimulate the animals to run. They ran 5 times a week regularly for 8 weeks altogether. The body weight was measured once a week. The sedentary control animals walked for 5 min on the treadmill at the same frequency as the trained animals. The animals were killed by heart puncture under phenobarbital anesthetization. The excised livers were cut into 1 g portions, frozen in liquid nitrogen and stored at −80 °C until use. The experiments were approved by the Committee for Laboratory Animals of our Faculty.

2.2. Isolation of nuclear DNA

One gram of the liver was homogenized in 6 vol. of a buffer containing 0.25 M sucrose, 50 mM Tris–HCl (pH 7.4) and 1 mM EDTA with a Teflon/glass homogenizer. The homogenate was layered on 5 ml of a cushion containing 0.35 M sucrose, 50 mM Tris–HCl (pH 7.4) and 1 mM EDTA in centrifuge tubes and centrifuged at 700*g* for 10 min at 4 °C. The fractions around the interface between the applied homogenate and the lower cushion were saved as crude mitochondria preparations (see next section). The nuclear pellets were suspended and nDNA extraction was performed as described previously in an argon atmosphere to prevent artificial oxidation of the DNA (Kaneko et al., 2004). Briefly, the pellet suspensions equivalent to 150 mg of the tissue were centrifuged and the resultant pellets were suspended in a buffer containing 1% Triton X-100, 0.3 M sucrose, 0.1% Desferal, 5 mM $MgCl_2$ and 10 mM Tris–HCl (pH 8.0). The pellets obtained after centrifugation were treated with proteinase K solution and the mixtures were centrifuged. The pellets containing DNA were treated with NaI and isopropyl alcohol. The crude DNA obtained by centrifugation was washed with isopropyl alcohol and ethanol. The final DNA pellets were dissolved in 0.01 × SSC, 1 mM EDTA and 10 mM Tris–HCl (pH 8.0) and the mixtures were incubated with RNase T1 and RNase A in the argon atmosphere. The solutions were treated with a chloroform and isoamyl alcohol mixture and centrifuged. The DNA in the aqueous phase was precipitated with 7 M NaI and isopropyl alcohol. The final DNA pellets were dissolved in distilled water treated with Chedex 100. The purity (absorption ratio at 260 and