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Effect of Endurance Exercise on the Tissue 8-Hydroxy-deoxyguanosine Content in Dogs

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The purpose of this study was to investigate the effect of endurance exercise on both the tissue and lymphocyte 8-hydroxy-deoxyguanosine (8-OHdG) content. Six dogs ran on a treadmill for 7 hours. Another six dogs were assigned to a sedentary control group. The exercised dogs were sacrificed immediately after exercise and the counterpart of the sedentary group was also sacrificed at the same time. The brain, lung, liver, spleen, kidney, jejunum, colon, diaphragm, heart, splenius muscle, and the medial and lateral portion of gastrocnemius muscle samples were then collected. Lymphocytes were sampled before and after exercise in the exercised dogs. The 8-OHdG content of lymphocyte DNA was found to significantly decrease after exercise (0.57 ± 0.19 vs 0.33 ± 0.10/deoxyguanosine (dG) $\times 10^5$, $P < 0.05$). The colon was the only tissue which showed a significant decrease in the content (0.83 ± 0.24 vs 0.54 ± 0.15/dG $\times 10^5$, $P < 0.05$). No tissue except for the colon showed any significant changes after exercise. These results therefore indicate that, immediately after endurance exercise, an augmented repair mechanism might thus play a role in the decrease of 8-OHdG in the lymphocytes and the colon, while the 8-OHdG generation might be counterbalanced by its repair in other tissues.

Keywords: Oxidative DNA damage, reactive oxygen species, lymphocyte, running

INTRODUCTION

Exercise is considered to be one of the physiological conditions in which the generation of reactive oxygen species (ROS) is augmented in the body.[1,2] Since the electron transport chain in the mitochondria is a major mechanism associated with ROS generation during exercise, it thus seems reasonable to assume that ROS generation would be augmented during endurance exercise.

It has been reported that ROS could harm DNA.[3–6] Regarding DNA damage caused by exercise-induced ROS, Alessio showed that the 8-hydroxydeoxyguanosine (8-OHdG)/creatinine ratio in urine increased 10 h after a marathon race.[7] The investigator suggested that, in severe exercise such as a marathon, DNA might be oxidized by exercise-induced ROS. Inoue *et al.* reported that the content of 8-OHdG in lymphocyte DNA significantly decreased immediately after a single bout of exercise, while the urinary excretion of 8-OHdG tended to increase, how-

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ever, the change was not significant.[8] They thus suggested that exercise stimulated the repair of oxidative DNA damage. The authors of the above mentioned studies thus hypothesized that exercise might enhance both the oxidative damage to DNA and the repair of oxidative DNA damage. However, the mechanism by which exercise affects the content of 8-OHdG in the DNA of various types of tissue remains to be elucidated.

The present study was therefore undertaken to investigate the effect of endurance exercise on the content of 8-OHdG in DNA in several different tissue specimens from dogs.

MATERIALS AND METHODS

Animals

Twelve beagle dogs (body weight 9.8 ± 0.9 kg, 14-months-old, CSK Research Park, Nagano, Japan) were used for the study. All animals were gradually acclimated to running on a treadmill before the experiment. The dogs were fed a commercial dry dog food (DS-5, Oriental Yeast, Tokyo, Japan) mixed with a commercial canned dog food (Pedigree, Master Food, Tokyo) at 16:00 every day.

Experimental Procedure

Six dogs were randomly assigned to an exercise group while the remaining 6 animals were assigned to a sedentary control group. The dogs in the exercise group ran on a treadmill at speeds ranging between 6 and 8 km/h. The running speed was selected according to the heart rate (HR) of each animal. The HR was maintained at about 200 bpm during the treadmill running. Because the HR max of beagle dogs during exercise was reported to be about 280 bpm[9] and the HR at rest in our study was about 80 bpm, the intensity of the exercise done in this study was considered to be about 60% of their HR reserve. All food was withdrawn at 17:00 on the day prior to the experiment and then treadmill running was started at 09:00. Each animal exercised for 7 hours (2 hours of running × 3 sets followed by another 1 hour run, each set was separated by 5 min of rest). Drinking water was allowed during the resting periods. The control animals were fasted and only allowed to drink water while the exercise counterpart was running. Blood samples were drawn both before and after the exercise from the saphenous vein of the exercised animals. Each animal was sacrificed with pentobarbital immediately after the exercise. The samples of the brain, the lung, the liver, the spleen, the kidney, the jejunum, the colon, the diaphragm, the heart, the splenius muscle, and the medial and lateral portion of gastrocnemius muscle were excised. The counterpart animals of the sedentary group were also sacrificed with pentobarbital at the same time as the exercised dogs and the same tissue samples were excised. The tissue samples were quickly frozen using an aluminum clamp in liquid nitrogen. All samples were stored at –80°C until performing the analysis of the 8-OHdG content in DNA. The content of the tissue and lymphocyte DNA was determined according to Yamamoto *et al.*[10] and Loft *et al.*[11] with some modifications.

Lymphocyte DNA Extraction

The lymphocytes were separated with LeucoPREP (Becton Dickinson, Lincoln Park, NJ). The DNA of the lymphocytes was extracted using a kit (DNA extractor WB, Wako Pure Chemical, Osaka, Japan). The method employed for both the lymphocyte and tissue specimens extracted the total DNA from the tissue. As a result, both nuclear and mitochondrial DNA were obtained.

DNA Extraction from Various Tissue Specimens

The tissue samples were homogenized with 2.5 ml of ice-cold 0.1 M EDTA in 0.15 M NaCl pH 8.0 using a Polytron homogenizer (Kinematica, Luzern, Switzerland) at 5,000 rpm for 3 seconds × 3 times. The homogenate was transferred to

another tube and then was centrifuged at 1,500 × g for 10 min at 4°C. After the supernatant was removed, 3 ml of the above mentioned buffer was added and mixed gently. Next a 1.5 ml portion was transferred to another tube and then 30 units of proteinase K (Boehringer Mannheim, Tokyo) were added. To each sample, 2 ml of 2% SDS in 0.1 M NaCl pH 8.0 were added. Each tube was filled with argon gas and heated for 30 min at 50°C. The air phase of each sample was replaced with argon gas at all following analytical procedures. After the addition of another 30 units of proteinase K, the samples were heated for 30 min at 50°C, then 0.4 ml of 2 M sodium acetate buffer and 8 ml of chilled isopropanol (–20°C) were added to precipitate DNA. The precipitated DNA was transferred to another tube using a pipette and then was washed with 4 ml of 70% ethanol. After the ethanol was removed, DNA dissolved in 1 ml of 1 mM EDTA was re-precipitated with 1.5 ml of chilled isopropanol, and then was washed with 1.5 ml of 70% ethanol. Finally, the ethanol was removed and the samples were dried under reduced pressure for 3 min.

DNA Enzymatic Digestion

The extracted DNA was dissolved in 100 µl of 1 mM EDTA and heated for 5 min at 95°C. Each sample was added with 1 µl of 2 M sodium acetate pH 4.5 and 50 µl of nuclease P1 (Sigma, St Louis, MO) then heated for 1 h at 37°C. To each sample 16 µl of 1 M Tris-HCl buffer pH 7.5 and 1.14 units of alkaline phosphatase (type III, Sigma) were added and then heated for 1 h at 37°C. The samples were centrifuged at 13,000 rpm for 3 min at 4°C and then the supernatant was used to measure 8-OHdG and deoxyguanosine (dG).

The Measurement of 8-OHdG and dG

The supernatant of 100 µ1 was applied to a LiChrospher 100 RP-18 column (4.0 mm × 150 mm, Merck, Darmstadt, Germany) and eluted with acetonitrile (1.5%, v/v) and methanol (1.5%, v/v) in 100 mM phosphate buffer pH 2.2 containing EDTA (5 mg/ml) at 1 ml/min. The amount of dG was determined from absorbance at 290 nm measured with a UV monitor (L-4250, UV-VIS detector, Hitachi, Tokyo). The amount of 8-OHdG in the lymphocyte DNA was measured with an ECD (Coulochem II, ESA, Bed Ford, MA) equipped with an analytical cell (model 5011, ESA, detector 1: 150 V, detector 2: 300 mV) and a guard cell (model 5020, ESA, 350 mV). The amount of 8-OHdG in DNA of the tissue samples was determined using an ECD (ECD-100, 750 mV, EICOM, Kyoto) fitted with a conditioning cell (model 5021, ESA, 70 mV). The content of 8-OHdG was expressed as the ratio of the peak area of 8-OHdG to the peak area $\times 10^5$ of dG. The standard 8-OHdG was prepared according to the procedure reported by Kasai *et al.* (4) while the standard dG was purchased from Wako Pure Chemical.

Statistics

When a one way analysis of variance showed significance, either the unpaired *t* test or Fisher's Protected Least Significant Difference test was used where appropriate. For a comparison of the lymphocyte content, the paired *t* test was used. Statistical significance was set at $P < 0.05$. All data represent the means ± SD.

RESULTS

The Content of 8-OHdG in the Lymphocyte DNA

The content of 8-OHdG in the lymphocyte DNA significantly decreased from $0.57 \pm 0.19/\text{dG} \times 10^5$ before exercise to $0.33 \pm 0.10/\text{dG} \times 10^5$ after exercise ($P < 0.05$).

The Content of 8-OHdG in DNA in the Different Tissue Specimens

Fig 1 depicts the values of the 8-OHdG content in the DNA from various types of tissue. Among

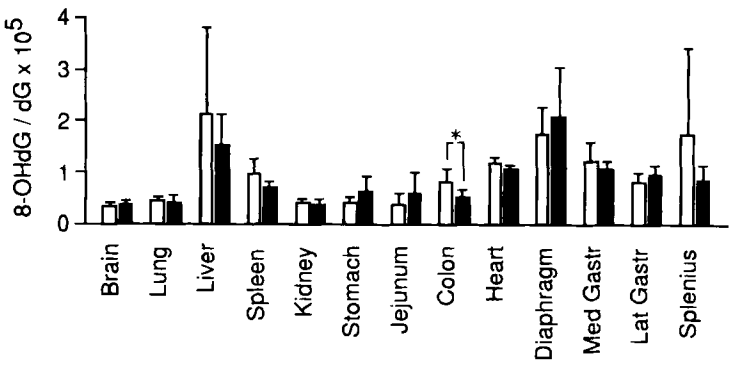

FIGURE 1 The 8-OHdG content in the DNA of several types of tissue before and after exercise. The values are the means ± SD for 6 animals. The open columns depict the values before exercise. The closed columns depict the values after exercise. *; $P < 0.05$, *t* test.

the examined tissue samples, the colon was the only tissue which showed a significant decrease in the 8-OHdG content after exercise (0.83 ± 0.24 vs $0.54 \pm 0.15/dG \times 10^5$, $P < 0.05$). No other tissue samples showed any significant changes after exercise.

The 8-OHdG content in the DNA from various types of tissue at rest varied among the tissue samples. The content of the liver ($2.14 \pm 1.67/dG \times 10^5$) was significantly higher than that of most other tissue specimens, except for the diaphragm ($1.75 \pm 0.52/dG \times 10^5$) and the splenius muscle ($1.77 \pm 1.66/dG \times 10^5$). The splenius muscle contained a significantly higher amount of 8-OHdG than the spleen ($0.98 \pm 0.30/dG \times 10^5$), the heart ($1.20 \pm 0.13/dG \times 10^5$), the diaphragm, and the medial gastrocnemius muscle ($1.22 \pm 0.40/dG \times 10^5$). The level of the diaphragm was significantly higher than that of the brain ($0.34 \pm 0.05/dG \times 10^5$), the lung ($0.45 \pm 0.09/dG \times 10^5$), the kidney ($0.41 \pm 0.09/dG \times 10^5$), the stomach ($0.42 \pm 0.10/dG \times 10^5$), the jejunum ($0.38 \pm 0.23/dG \times 10^5$), the colon ($0.83 \pm 0.24/dG \times 10^5$), and the lateral gastrocnemius muscle ($0.82 \pm 0.17/dG \times 10^5$), respectively. The heart 8-OHdG level was significantly higher than that observed in both the brain and the jejunum. The medial gastrocnemius also showed a significantly higher content compared with the brain, the kidney, and the jejunum.

DISCUSSION

In this study, the content of 8-OHdG in the lymphocytes also significantly decreased after exercise. This finding is consistent with the results of Inoue *et al.*.[8] They speculated that the decrease in the content of 8-OHdG might be due to an augmented repair of the oxidative DNA damage. In the present study, we also observed a significant decrease in the content of 8-OHdG in the DNA of the colon. The mechanism for the decrease is not clear, however, the increased elimination of 8-OHdG from the DNA reported in the lymphocytes (8) is thus considered to be associated with the decrease observed in the colon tissue as well. With regard to the other tissue specimens investigated in the present study, no significant changes were observed. The mean values of the liver and splenius muscle were lower, but not significantly so, in the exercised animals than the control ones. Since the values of these tissue specimens from the control animals showed a high variability, we could not rule out the possibility that the effect of exercise itself decreased the 8-OHdG content in these tissue specimens. The ratio of urinary 8-OHdG/creatinine, which is an indicator of damaged and/or repaired DNA previously injured by ROS, was reported to increase 10 h after a marathon race.[7] Inoue *et al.* also observed that the urinary excretion of 8-OHdG

also tended to increase. In this study, the dogs urinated during exercise, however, because of the difficulty of collecting urine during running, we could not measure the urinary excretion of 8-OHdG. It thus seems possible that the urinary excretion of this substance increased during exercise.

It was reported that increases in the serum enzymes were more prominent 4 h after exercise compared to immediately after.[12] This finding thus suggests that the content of 8-OHdG in the tissue specimens would have been higher if tissue samples had been taken later rather than immediately after exercise. The finding that the level of malondialdehyde in the liver was higher 48 h after exercise than immediately after exercise in rats[13] also supports this hypothesis. The time course of the changes in the 8-OHdG content in the DNA of different types of tissue after exercise therefore still needs to be further investigated.

Regarding the difference in the content of 8-OHdG among the various types of tissue, Fraga *et al.* measured the content in the brain, the testes, the liver, the kidney, and the intestine.[14] They found that the kidney showed the highest content, thus suggesting that the high oxygen consumption of the organ might account for the highest content among all the tissue specimens measured. Loft *et al.* showed that the amount of 8-OHdG excreted into the urine correlated to oxygen consumption in humans.[15] In addition, the oxidative damage to DNA was reported to increase in proportion to the species-specific basal metabolic rate.[16,17] These findings thus support the hypothesis that the content of 8-OHdG in the DNA of various types of tissue might depend on the oxygen consumption of each type of tissue. In the present study, however, the content of 8-OHdG in the kidney at rest was lower than that in the liver and the value in the kidney did not differ from that observed in the brain or the intestine. In addition, in the present study, the liver showed the highest content. Regarding the oxygen consumption of the different tissue specimens,[18] the liver (2.0 ml/100g/min) consumed less oxygen than the kidney (6.0 ml/100g/min), the brain (3.3 ml/100g/min), or the heart muscle (9.7 ml/kg/min). In addition, in spite of a lower oxygen consumption (0.2 ml/100g/min), the content of 8-OHdG in the skeletal muscle was higher than all examined tissue samples except for the liver in the present study. These results suggest that the oxygen consumption of a tissue may not be the only factor determining the content of 8-OHdG. The reason for the difference between our results and those of Fraga *et al.*,[14] however, remain unclear.

In conclusion, the content of 8-OHdG in the DNA of both lymphocytes and colon tissue decreased after exercise, thus suggesting that the elimination of 8-OHdG was augmented during exercise. The content of 8-OHdG in the other examined tissue specimens, however, showed no significant changes, thus suggesting that the rate of elimination would be counterbalanced by its generation. Future studies focusing on the time course of the content of 8-OHdG in DNA after exercise are thus still needed to elucidate the effect of exercise on the oxidative damage to DNA.

References

[1] K. J. A. Davies, A. T. Quintanilha, G. A. Brooks and L. Packer (1982). Free radicals and tissue damage produced by exercise. *Biochemical and Biophysical Research Communications*, **107**, 1198–1205.

[2] C. J. Dillards, R. E. Litov, W. M. Savin, E. E. Dumelin and A. L. Tappel (1978). Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. *Journal of Applied Physiology*, **45**, 927–932.

[3] E. S. Fiala, C. C. Conway and J. E. Mathis (1989). Oxidative DNA damage in livers of Sprague-Dawley rats treated with the hepatocarcinogen 2-nitropropane. *Cancer Research*, **49**, 5518–5522.

[4] H. Kasai and S. Nishimura (1984). Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Research*, **12**, 2137–2145.

[5] H. Kasai, S. Nishimura, Y. Kurokawa and Y. Hayashi (1987). Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. *Carcinogenesis*, **8**, 1959–1961.

[6] C. Richter, J. W. Park and B. N. Ames (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 6465–6467.
[7] H. M. Alessio (1993). Exercise-induced oxidative stress. *Medicine and Science in Sports and Exercise*, **25**, 218–224.
[8] T. Inoue, Z. Mu, K. Sumikawa, K. Adachi and T. Okochi (1993). Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. *Japanese Journal of Cancer Research*, **84**, 720–725.
[9] G. C. Haidet (1989). Dynamic exercise in senescent beagles: oxygen consumption and hemodynamic responses. *American. Journal of Physiology*, **257**, H1428–H1437.
[10] F. Yamamoto, H. Kasai, Y. Togashi, N. Takeichi, T. Hori and S. Nishimura (1993). Elevated levels of 8-hydroxydeoxyguanosine in DNA of liver, kidneys, and brain of Long-Evans cinnamon rats. *Japanese Journal of Cancer Research*, **84**, 508–511.
[11] S. Loft, K. Vistisen, M. Ewertz, A. Tjonneland, K. Overvad and H. E. Poulsen (1992). Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*, **13**, 2241–2247.
[12] R. J. Maughan, A. E. Donnelly, M. Gleeson, P. H. Whiting, K. A. Walker and P. J. Clough (1989). Delayed-onset muscle damage and lipid peroxidation in man after a downhill run. *Muscle and Nerve*, **12**, 332–336.
[13] L. L. Ji, F. W. Stratman and H. A. Lardy (1988). Enzymatic down regulation with exercise in rat skeletal muscle. *Archives of Biochemistry and Biophysics*, **263**, 137–149.
[14] C. G. Fraga, M. K. Shigenaga, J. W. Park, P. Degan and B. N. Ames (1990). Oxidative damage to DNA during against aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 4533–4537.
[15] S. Loft, A. Astrup, B. Buemann and H. E. Poulsen (1994). Oxidative DNA damage correlates with oxygen consumption in humans. *The FASEB Journal*, **8**, 534–537.
[16] R. R. Adelman, R. L. Saul and B. N. Ames (1988). Oxidative damage to DNA: Relation to species metabolic rate and life span. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 2706–2708.
[17] M. K. Shigenaga, C. J. Gimeno and B. N. Ames (1989). Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 9697–9701.
[18] W. F. Ganong (1989). Circulation through special regions. In *The Review of Medical Physiology*, Lange, Norwalk, Connecticut, p. 514.