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Rapid Letter

Attenuation of the Development of Murine Solid Leukemia Tumor by Physical Exercise

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ABSTRACT

The active involvement of physical exercise in the evolution of a variety of cancers is well documented. However, its role in solid leukemia tumor development is essentially unknown. Solid leukemia tumor cells were transplanted into 21 hybrid BDF1 control mice, exercise-trained mice that did not exercise during leukemia and exercise-trained mice that exercised during leukemia. The tumor size of the continuously exercising group was ~50% of that of control and exercise-terminated animals 18 days after the transplantation. The activity of antioxidant enzymes and the levels of lipid peroxidation and 8-hydroxy-2′-deoxyguanosine were not different in the tumors of the three groups. The level of carbonylated proteins was smaller in tumors of continuously exercising animals. The mutant form of cell regulatory protein p53 and vascular endothelial growth factor were present in similar amounts in the tumor cells of each group. On the other hand, the protooncogene Ras and I-κB proteins were present in higher concentrations in tumors of continuously exercising rats. The present data suggest that exercise during leukemia attenuates the development of tumors in mice. The selective alteration of regulatory proteins might play a role in the beneficial effects of exercise during leukemia. Antioxid. Redox Signal. 4, 213–219.

INTRODUCTION

THE DEVELOPMENT OF CANCER is determined by a combination of heredity and environment. However, the incidence and progress of the disease are most probably dependent upon the function of health-promoting genes that produce the correct synthesis of proteins to sustain the viability of cells and maintain or increase the resistance to damage and damage repair processes. Evidence has been accumulating that indicates that regular physical exercise significantly reduces the incidence of cancer. The beneficial effect can be as high as 50% for a variety of cancers (10, 15, 29, 31). Chronic physical exercise may decrease risk by affecting natural immunity, including the rate of cytokine production, hormonal changes, and antioxidant defense, or by improving energy balance (5, 6).

Oxidative damage to DNA may also underpin certain cancers, resulting in the loss of function of tumor-suppressor genes and activation of tumor promoting genes with subsequent malignancy (1, 20). Our recent study led us to suggest that physical exercise could be an active tool against cancer (25). We found that as a result of 9 weeks of swimming

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the level of DNA damage, measured as 8-hydroxy-2′-deoxyguanosine (8-OHdG) content, significantly decreased in exercised rats compared with control animals. The mutagenic effect of 8-OHdG is well documented (9). The attenuated level of 8-OHdG due to exercise training might be accounted for by the decreased rate of production of reactive oxygen species (ROS) in the mitochondrial respiration in state 3 and a drop in mitochondrial membrane potential (14, 35).

In addition to epidemiological data, experimental results indicate that, indeed, regular exercise retards the development of certain cancers (31, 32). Several hypotheses have arisen to explain this phenomenon involving the beneficial effects of exercise on the hormonal system (5), on energy metabolism (32), and on the antioxidant system (5). It seems to be a reasonable assumption that exercise-induced beneficial effects on cancer development and incidence are not due to one particular pathway, but more likely are the result of several mechanisms that somehow are altered by regular exercise. The purpose of the present investigation was to indentify some ROS-related mechanisms by which regular exercise could affect the development (not the incidence) of leukemia solid tumor. Leukemia tumor was selected because the environmental effects on the incidence of the disease are significant (23), and because leukemia is associated with marked ROS production and redox processes (30).

We hypothesized that the ROS-associated changes in the accumulation of 8-OHdG, reactive carbonyl derivatives (RCD), lipid peroxidation (LIPOX), and/or the concentration of redox-sensitive regulatory proteins play a role in the exercise-induced alteration of tumor development.

MATERIALS AND METHODS

Animals

Twenty-one first-generation hybrid BDF1 (C57B1/6 female and DBA male from our animal house) adult female mice, weighing 23–25 g, specified pathogen-free, were used in the study. The animals were kept on a 12-h light/dark cycle and fed with a sterilized standard diet (Biofarm, Budapest, Hungary) and tap water *ad libitum.*

Tumor and exercise

Murine P-388 lymphoid leukemia obtained from the Biological Testing Branch of the National Cancer Institute was maintained in live BDF1 mice by several passages *in vivo* and inoculated subcutaneously with 5×10^6 cells per mouse. The mice were assigned to three groups: control (C); exercised trained (ET) with the exercise being terminated at the point of tumor-cell transplantation; and exercised trained with the animals continuing exercise (EC) throughout the experiment. Exercised mice had five swimming training sessions per week that lasted for 1 h for 10 weeks. This amount of weekly swimming has been demonstrated to result in significant reduction of hepatoma tumor in rats (2). The exercise session was done at the same time, and the water temperature was set to 32°C degree. Leukemic cells were transplanted into the mice, and the tumor dimensions were measured every 2 days using a microcaliper. Volume of the tumor was calculated as described by Tomayko and Reynolds (33) with the following formula: $V = \pi/6 \times L \times D^2$, where V = tumor volume, L = longest diameter, and D = diameter perpendicular to L. The EC group continued the swimming training until the termination of the study, which was on the 18th day following transplantation. The animals were killed, tumor samples were collected into liquid nitrogen, and then tumors were analyzed.

Chemicals

All chemical reagents were obtained from Sigma, except where noted. Antibodies for I-κB and vascular endothelial growth factor (VEGF) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.), and Ras from Stress-Gen Biotechnologies Corp. (Canada, catalog no. KAP-GP001E). The mutant p53 protein ELISA was purchased from Calbiochem (Schwabach, Germany) and measured according to the supplier (catalog no. QIA 03). The

proteasome RC2 subunit antibody was obtained as described previously (13).

Assays

The activity of superoxide dismutase (SOD) was determined according to the method of Mishra and Fridovich (21). Mitochondrial Mn-SOD activity was measured by adding potassium cyanide because the cyanide inhibits Cu,Zn-SOD activity, but not Mn-SOD. Cu,Zn-SOD activity was calculated by subtracting Mn-SOD activity from total SOD activity. The activity of glutathione peroxidase (GPX) was assayed by the method of Sedlak and Lindsay (27). The activity of catalase was measured accordingly to Beers and Sizer (3). For the estimation of malondialdehyde, a lipid peroxidation marker, the thiobarbituric acid-reactive substances (TBARS) were determined by the method of Uchiyama and Mihara (34). The oxidative modification of amino acid residues was measured by the accumulation of RCD as described previously (24). Citrate synthase activity (CS) was analyzed as a marker of an aerobic training effect in skeletal muscle (24). The free radical induced modification of nuclear DNA was measured by HPLC with electrochemical detection as previously described (17). Western blotting was carried out as described earlier (24) to measure the protein content of I-κB, VEGF, and Ras. The data were quantified by densitometry and expressed in arbitrary units.

Statistical analysis

Results are presented as means ± SD. Differences between groups were compared using Student's t test. Multiple comparisons were performed using a one-way analysis of variance. Significance was set at $p < 0.05$.

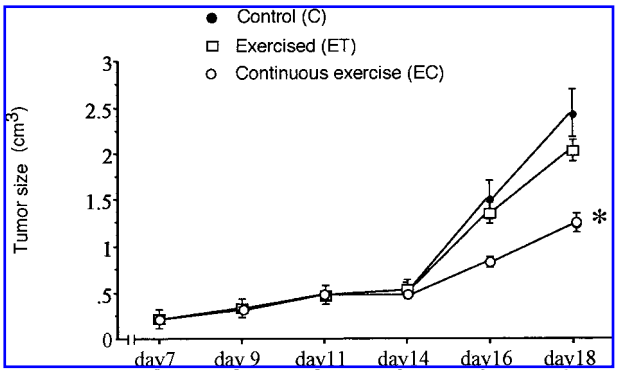

FIG. 1. Lymphoid leukemia cells (5×10^6 were subcutaneously inoculated in mice. The tumor size of continuously exercising animals was significantly smaller on the 18th day, following the transplantation, compared with control and exercise-terminated groups. Values are means ± SD of seven animals. *$p < 0.05$, C vs. EC.

RESULTS

The tumor size of EC animals was significantly smaller than that of the C and ET groups ($p < 0.05$), and the development of the tumor was especially different during the last 4 days (Fig. 1). Exercise, but not exercise history, depressed the size of solid leukemia tumor. The swimming regimen significantly increased CS activity from 0.19 ± 0.01 (C) to 0.32 ± 0.02 (ET) and 0.38 ± 0.02 mol/mg of protein (EC) ($p < 0.05$), and this indicates an aerobic training effect on skeletal muscle. There were no differences in the activities of

TABLE 1. ANTIOXIDANT ENZYME ACTIVITIES IN TUMOR CELLS

	Control (C)	*Exercised (ET)*	*Continuous exercise (EC)*
Mn-SOD	826 ± 70	759 ± 55	903 ± 90
Cu,Zn-SOD	1,872 ± 240	2,075 ± 230	1,974 ± 210
GPX	19.7 ± 1.15	22.1 ± 1.42	18.75 ± 1.67
Catalase	0.03 ± 0.002	0.026 ± 0.001	0.027 ± 0.001

The activity of antioxidant enzymes was not significantly different in the different sizes of tumors. Values are means ± SD for six rats each group and are expressed as U/mg of protein.

TABLE 2. OXIDATIVE DAMAGE OF MACROMOLECULES IN SOLID LEUKEMIA TUMOR

	Control (C)	*Exercised (ET)*	*Continuous exercise (EC)*
8-OHdG/10^{-5} dG	0.27 ± 0.02	0.23 ± 0.02	0.23 ± 0.03
Carbonyl (nmol/mg of protein)	7.69 ± 0.71	7.58 ± 0.79	5.62 ± 0.89*
TBARS (nmol/mg of protein)	0.94 ± 0.06	1.17 ± 0.18	1.20 ± 0.06

The level of carbonyl groups decreased significantly in tumors of EC animals compared with controls. Values are means 6 SD for six rats each group.
*Significantly different from control and exercised groups ($p < 0.05$).

antioxidant enzymes, LIPOX, and DNA damage (Tables 1 and 2). The levels of RCD were lower in tumors of EC than those of ET and C ($p < 0.05$)(Table 2). The protein content of proteasome and mutant p53 protein was not different in any of the tumors (Table 3). On the other hand, densitometric data revealed that Ras protein was present in higher concentrations in tumor cells of EC, which had smaller tumors, than C (Fig. 2). Similarly, the nuclear transcription factor inhibitor I-κB protein concentration was elevated in the tumor cells of EC compared with C and ET (Fig. 2). The protein concentration of VEGF tended to be lower in tumor EC than C animals, but the difference did not reach statistical significance (Table 3).

DISCUSSION

Regular exercise during leukemia decreased tumor size by nearly 50%. Interestingly, the tumor size of ET animals was comparable to that of controls. These data suggest that exercise does have the capability to attenuate the development of tumors by some as yet unknown mechanism. Free radicals might be involved in the regulatory process of cell proliferation (18, 28) via oxidative damage-mediated mechanisms and/or redox signaling. The activity levels of antioxidant enzymes were not altered, but the levels of RCD were smaller in tumors of EC animals. The RCD levels were relatively high in tumors of all groups, indicating an inefficient removal of oxidized proteins (the RCD levels in our laboratory generally range between 0.5 and 1.5 nmol/mg of protein). Low levels of RCD in smaller tumors could mean lower levels of ROS production or efficiently functioning proteasome complex. The levels of LIPOX tend to increase in smaller tumors, which suggests that the lower concentration of RCD might be due to the active proteasome rather than lesser levels of ROS. However, the protein concentration of proteasome was similar in all groups, despite the differences in tumor size. Increased levels of LIPOX could curb proliferation, and hence low LIPOX levels in

TABLE 3. MUTANT P53, VEGF, AND PROTEASOME PROTEIN CONTENT IN SOLID LEUKEMIA TUMOR

	Control (C)	*Exercised (ET)*	*Continuous exercise (EC)*
Mutant p53 (ng/mg of protein)	0.26 ± 0.08	0.24 ± 0.05	0.20 ± 0.07
VEGF (arbitrary unit)	3.67 ± 0.61	3.31 ± 0.63	2.7 ± 1.12
Proteasome (arbitrary unit)	7.17 ± 0.84	6.97 ± 1.71	7.34 ± 1.35

The different tumor sizes of the animals were not associated with significantly different levels of mutant p53, VEGF, and proteasome content. Values are means ± SD for six rats in each group.

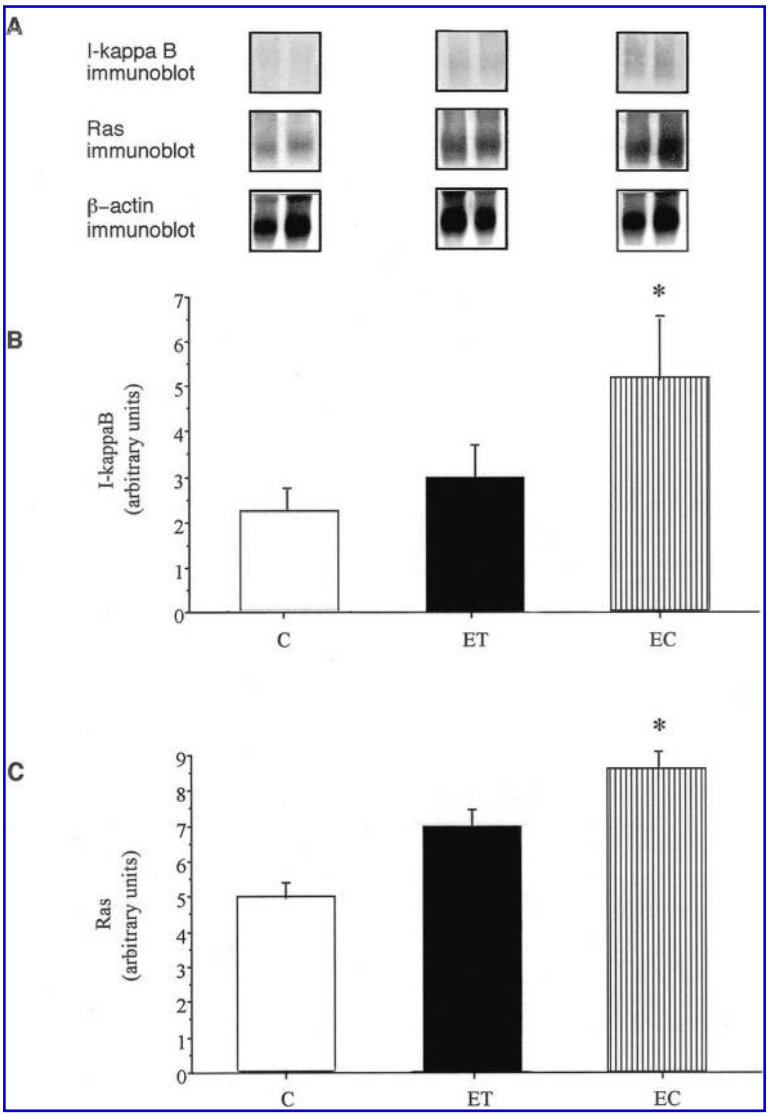

FIG. 2. (**A**) The immunoblot data of I-κB, Ras, and β-actin, which was loaded as a control. (**B** and **C**) The densitometry data of immunoblot signals revealing significant differences between C and EC samples. Smaller tumors were associated with higher amounts of I-κB and Ras protein content, indicating a difference in redox signaling pathway and link between redox signaling and tumor development. Values are means ± SD of seven animals. $^{*}p < 0.05$, C vs. EC.

large tumors do not seem to be a paradox (10). It cannot be excluded that the well documented accuracy problems in LIPOX measuring methods might effect the LIPOX data (22); however, plasma LIPOX levels of leukemia patients also were in the normal range (7).

In the present study, we did not observe significant free radical formation as estimated by the activity of antioxidant enzymes and oxidative damage. However, the physiological concentration of free radicals can act through the induction of those proteins that are sensitive to redox changes in the cell milieu. Therefore, we measured the protein content of certain cell regulatory proteins, such as p53, I-κB, and Ras. The wild type of p53 is considered to be an antioncogene product (8, 19), while point mutations cause accumulation of mutant p53, especially in tumors (4). The data suggest that the differences in tumor growth were not due to the different contributions of p53 and proteasome in the apoptosis, because these agents can play a significant role in programmed cell death (11). Ras, a protooncogene protein, binds and hydrolyzes GTP and may be distantly related to the G proteins involved in the cell transduction (18). Ras protein could trigger downstream mitogen-activated protein kinase cascades, resulting in nuclear factor-κB (NF-κB) activation (16). However, in the present study,

elevated Ras protein concentration was present in smaller tumors, indicating possible increased NF-κB activation and hence a higher rate of I-κB degradation. This might indicate that NF-κB activation was due to other signaling pathways, or it could also mean that I-κB protein concentration is not always a valid method to assess NF-κB activation.

Upon activation of NF-κB, which was observed by a wide variety of agents, stressors, oxidants, etc., the I-κB was phosphorylated and degraded by 26S proteasome. Inhibition of phosphorylation could prevent the activation of NF-κB (16). Therefore, the protein content of I-κB indicates the rate of NF-κB translocation and the rate of transcription. I-κB and NF-κB dissociation is achieved by ubiquitination and proteasome-mediated breakdown of I-κB α (12).

Higher levels of I-κB in smaller tumors might indicate a lower level of dissociation and transcription, and thereby as a result an attenuated cell growth. The data from this study do not exclude the possibility that the attenuated rate of tumor development in continuously exercised animals might be mediated by direct free radical interactions on cell-signaling proteins. We suggest that exercise during leukemia has the capability to attenuate the development of solid tumors. However, the mechanism of control still remains vague. The generation of free radical species seems to be moderate in solid leukemia tumors, because it does not induce the activity of antioxidant enzymes and significant oxidative damage. Similar data have been obtained on sarcoma and colon tumor-bearing mice, suggesting that physical exercise could retard the development of different types of cancer (26; Radak *et al.*, unpublished observations). Leukemia is known to modify redox pathways (30), and it cannot be excluded that exercise-induced alteration of redox-sensitive proteins like Ras and I-κB are associated with retardation of tumor growth. The importance of the evidently complex underlying mechanisms, with respect to cancer incidence and tumor development-retarding effects of regular exercise, warrants further investigation.

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ABBREVIATIONS

C, control; EC, exercised during leukemia; ET, exercise trained; GPX, glutathione peroxidase; LIPOX, lipid peroxidation; NF-κB, nuclear factor-κB; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; RCD, reactive carbonyl derivatives; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; VEGF, vascular endothelial growth factor.

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