Effects of moderate exercise and oat β-glucan on lung tumor metastases and macrophage antitumor cytotoxicity

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FROM A PUBLIC HEALTH perspective, both diet and exercise are usually the focus of behavioral changes suggested to reduce the risk for some of the major site-specific cancers (12, 13). Both epidemiological and animal experiments provide insight into the links between diet and cancer prevention (13). Dietary fiber, vegetables and fruits, micronutrients, and phytochemicals have all been investigated for their anti-tumor potentiating activity that usually involves immune system mechanisms (13). Lack of exercise has been one of the most consistently identified risk factors for all cancer mortality (12, 37), as well as site-specific cancers such as colorectal cancer (4) and breast cancer (19). However, the strength of the association remains questionable due to the lack of data from controlled experimental studies and the fact that specific mechanisms have not been elucidated. The amount and intensity of exercise required to reduce the risk of cancer have also not been determined.

Limited evidence from animal models typically demonstrates that exercise inhibits the incidence and progression of cancer in a variety of tumor models, including chemically induced tumor models (30, 31, 33), genetically predisposed models (3), and implantation/transplantation models (6, 24), although not in all cases (32, 39). Differences in the intensity and duration of exercise, the timing of exercise in relation to administration of tumor cells or carcinogen, and specific tumor model are likely to contribute to the inconsistencies.

The role of exercise in tumor metastases, perhaps the most devastating aspect of cancer, has not been widely studied. The lung is one of the major sites of metastases. A recent study in our laboratory found a decrease in the number of lung tumor foci in mice after intravenous administration of the B16 melanoma cells after a single bout of prolonged running on a treadmill (6). Similarly, 9 wk of wheel-running activity in mice before administration of CIRAS 1 tumor cells resulted in a lower distribution of tumor metastases in the lungs (22). The mechanisms underlying the exercised-induced inhibition of experimental tumor metastasis are not well understood. Components of the innate immune system can play an important role in limiting metastases by altering the early steps of implantation and growth of secondary foci (1, 11) of various cancer cells, including B16 melanoma. Macrophages and natural killer (NK) cells are cytotoxic to a number of tumor cells in vitro as well as in vivo (6, 11, 17, 18, 21). Exercise-induced increases in the anti-cancer functions of these cells have been reported (6, 17, 18, 20, 21, 23, 40, 41). Many other potential mechanisms have also been investigated, including steroid hormones, oxygen free radicals, insulin and insulin-like growth factors, as well as energy balance and body composition, but they are typically investigated in other cancer models involving tumorigenesis that develops over long periods (35).

The dietary fiber β-glucan, derived from the cell wall of yeast and fungi, has well-documented effects in cancer prevention in a variety of syngenic murine tumor models, including B16 melanoma, adenocarcinoma, mammary carcinoma, lymphocytic leukemia, and Lewis lung carcinoma, presumably acting via activation of both nonspecific and specific immune mechanisms (8, 27). β-Glucan exerts its effects through direct stimulation of macrophage, neutrophil, and NK cells via β-glucan-specific receptor sites on their cell surface membranes (5, 34), such as complement receptor 3 and dectin-1 (2). The exact mechanisms are at least partially dependent on the route of administration. Protection after oral administration results primarily from ingestion of small particles of β-glucan by pino-
cytic M cells located in Peyer’s patches of the small intestine (9). Once activated, these cells can migrate to the lymph nodes and are capable of activating other macrophages, NK cells, and T lymphocytes via the release of cytokines (25, 26, 28). Soluble β-glucan from oats is beginning to receive greater attention due to its recognition by the Food and Drug Administration as part of the Heart Healthy Diet and its well-documented health benefits in other pathological conditions, including diabetes and cardiovascular disease (16, 42). However, there have been no reports on the specific benefits of soluble oat β-glucan on any form of cancer.

The purpose of this study was to determine the effects of short-term moderate-exercise training and oral feedings of soluble oat β-glucan on the metastatic spread of injected tumor cells. The effects on macrophage cytotoxicity were also studied as a possible mechanism. This was done by using an experimental murine model of lung metastases involving intravenous administration of B16 melanoma cells in which our laboratory has previously shown a benefit of a single run to fatigue (~2.5 h), but not a 30-min run, on lung tumor foci (6). However, the exercise protocol was modified for this experiment to include 6 consecutive days of treadmill running (1 h/day) to better assess the role of short-term moderate-exercise training. This exercise protocol can increase macrophage anti-viral function and reduce the risk of respiratory viral infection in mice (7). We hypothesized that both exercise and oat β-glucan would decrease the metastatic spread of injected tumor cells, which would be associated with an increase in macrophage cytotoxicity. Furthermore, we hypothesized that there would be an additive effect of exercise and oat β-glucan.

METHODS

Animals. Male C57BL/6 mice, 6 wk of age, were purchased from Harlan Sprague-Dawley Laboratories and acclimated to our facility for at least 3 days before any experimentation. Mice were purchased as pathogen-free stock, and periodic screening of sentinel mice yielded negative results for common murine viral or bacterial pathogens. Mice were housed four per cage and cared for in the animal facility at the University of South Carolina Medical School. Mice were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% humidity, low noise) and given food (Purina Chow) and water (or oat β-glucan dissolved in water) ad libitum. Separate groups of mice were used for each dependent variable: in vivo lung tumor metastases (n = 8–11 per group) and peritoneal macrophage cytotoxicity (n = 8 per group). All experiments were performed at the end of the active dark cycle.

Nutrient treatment. Mice were randomly assigned to one of the following four groups: exercise water (Ex-H2O), exercise oat β-glucan (Ex-OβG), control water (Con-H2O), or control oat β-glucan (Con-OβG). Ex-H2O and Con-H2O received tap water for the 10 days before tumor administration or death, whereas Ex-OβG and Con-OβG mice were fed a solution of oat β-glucan dissolved in drinking water for the 10 days before tumor administration or death and the 14 days after tumor administration. The oat β-glucan solution was made from an oat bran concentrate enriched to 68% soluble β-glucan (manufactured by Nurture, Devon, PA, and supplied by Quaker Oats, Barrington, IL), which was dissolved in the drinking water at a concentration of 0.6 mg/ml and made fresh daily. The remainder of the oat bran concentrate included starch, as well as small quantities of protein, sodium, and fat. Soluble oat β-glucan is a structural polysaccharide (~2 × 105 molecular weight) found in the cell walls of the bran layer and endosperm fractions of the whole seed. Structurally, they are linear chains of β-D-glucopyranosyl units in which ~70% of the units are linked (1–4), but which also consist of β-D-cellotriosyl and β-D-cellotetraosyl residues separated by (1–3) linkages arranged in a random manner (36). Daily consumption of fluid was measured to ensure that there were no differences in fluid ingestion between the water and the oat β-glucan solution. Body weight of each animal was monitored throughout supplementation and exercise period to ensure that no weight loss was experienced by any group.

Treadmill acclimation and exercise protocol. The University’s Institutional Animal Care and Use Committee approved the protocol described. Beginning on the second and day of oral β-glucan or water treatment, exercise mice (Ex-H2O and Ex-OβG) were acclimated to the treadmill for a period of 20 min a day for 3 consecutive days. The exercise protocol consisted of a 1-h bout of treadmill running (performed in the morning, 7 AM) for 6 consecutive days. Mice ran on the treadmill (2 per lane) at a speed of 36 m/min and a grade of 8%, which is estimated to elicit ~75–90% maximal O2 uptake (10, 29), assuming a maximal O2 uptake of 173–206 ml·kg−1·min−1 for mice. Male C57BL/6 mice are capable of running for ~2.5 h at this exercise intensity in our hands (6). Electric shock was never used in these experiments, as mice readily respond to a gentle tap of the tail or hindquarters, encouraging them to maintain pace with the treadmill. Mice rarely require this type of continual prodding during the 1-h exercise bout. Mice in the control groups (Con-H2O and Con-OβG) remained in their cages in the treadmill room through the exercise bouts. These mice were exposed to similar handling and noise in an attempt to control for extraneous stresses that may be associated with treadmill running. Control mice were deprived of food and water during the exercise sessions.

Tumor metastases. The tumor cell line B16Fl, melanoma (ATCC no. CRL 6323; American Type Culture Collection, Rockville, MD), which is syngeneic to the C57BL/6 mouse strain, was used in all experiments. Tumor cells were maintained in RPMI media (GIBCO BRL, Grand Island, NY) supplemented with 10% fetal bovine serum and 2% penicillin, streptomycin, and L-glutamine. The adherent B16 cells were removed from tissue culture flasks by incubating them for 10 min with trypsin-EDTA (GIBCO BRL). Cells were harvested by centrifugation, washed once, and adjusted to concentrations for specific experiments. To assess the development of pulmonary metastases, 0.2 ml of B16 melanoma cells (1 × 106 cells/ml) were injected into a tail vein. Mice (n = 8–11 per group) were injected 30 min after the last day of exercise or rest. After tumor administration, mice were returned to their cages and remained in the animal facility. Mice continued on the oat β-glucan treatment during this period, but exercise stopped. Mice were killed 14 days after tumor administration. The lungs were removed at death and stained with Bouin’s fixative. The number of tumor foci (pulmonary metastases) on the surface of the lungs was counted under a dissecting microscope by an investigator blinded to the treatments.

In vitro peritoneal macrophage cytotoxicity. After the last day of exercise or rest, mice (n = 8 per group) were euthanized by halothane overdose. Death occurred within 1 min. Peritoneal macrophages were obtained by lavage of the peritoneal cavity with 5 ml of media. The culture medium used was RPMI-1640 (GIBCO BRL). Peritoneal lavage cells were washed once in RPMI-1640, and any remaining red blood cells were lysed with tris(hydroxymethyl)-aminomethane-ammonium chloride, pH 7.2. Peritoneal cells were seeded in 96-well flat-bottom microtiter plates at several concentrations (4 × 103, 2 × 103, 1 × 103, and 5 × 103) to elicit effector target concentrations of 80:1, 40:1, 20:1, and 10:1. Cells were maintained at 37°C and 5% CO2 in a random manner (36). Daily consumption of fluid was measured to ensure that there were no differences in fluid ingestion between the water and the oat β-glucan solution. Body weight of each animal was monitored throughout supplementation and exercise period to ensure that no weight loss was experienced by any group.

The plates were incubated for 48 h at 37°C without macrophages. The plates were then incubated for 48 h at 37°C with 1 μCi of [3H]thymidine (ICN Biomedicals, specific activity 6.7 Ci/mmol) were added to each well. The plates were incubated for 24 h, and the amount of TdR incorporated was determined by scintillation counting.
Tdr uptakes in the wells containing only B16 tumors were used as control values. This uptake measured “uninhibited” growth of the tumor cells; therefore, any Tdr uptake less than this represents cyostasis or cytolysis of the tumor cells by the macrophages. Percent cytoxicity was calculated as follows: (Tdr uptake in test well/Tdr uptake in control well) × 100. Macrophages do not proliferate and incorporate Tdr in this assay. Therefore, tumor cell growth is quantified by measuring Tdr uptake. This assay measures both macrophage-mediated tumor growth inhibition (cyostasis) and cytolysis, because lysed cells do not incorporate Tdr.

Statistical analysis. Statistical analyses were performed by using a commercially available statistical package from SigmaStat (version 2.03, SigmaStat, SPSS, Chicago, IL). Fluid consumption, lung tumor foci, and macrophage cytoxicities were analyzed by two-way ANOVA (exercise × oat β-glucan) with Student Newman-Keuls post hoc analysis (P < 0.05).

RESULTS

Nutrient consumption. There were no differences in the average amount of fluid consumed by each group. Over the course of the fluid treatment, Con-H2O mice consumed an average of 4.5 ± 0.21 ml/day, which was similar to Ex-H2O (4.8 ± 0.60 ml/day), Ex-OB, G (5.1 ± 0.42 ml/day), and Con-OB, G (4.8 ± 0.15 ml/day). Therefore, 24-h fluid consumption was not affected by the dissolved oat β-glucan or moderate exercise. This is also reflected by a lack of difference in body weight across the groups. Weight gain over the course of the acclimation phase and 6-day exercise period was 0.95 ± 0.56 g in Ex-H2O, 0.82 ± 0.38 g in Ex-OβG, 0.8 ± 0.44 g in Con-H2O, and 0.64 ± 0.74 g in Con-OβG. Food intake was not measured in this study, but it is unlikely that significant differences occurred given the similar body weights across groups.

Pulmonary metastases. The number of lung tumor foci was measured at four effector-to-target ratios, as seen in Fig. 2. There were no differences in the number of lung tumor foci after injection of B16 melanoma cells. Figure 1 illustrates the spread of injected B16 melanoma cells. There were significant main effects for both exercise (P < 0.05) and oat β-glucan (P < 0.05); however, there were no interactions. Tumor counts were as follows: 95 ± 12.0 in Ex-H2O, 102 ± 12 in Ex-OβG, 189 ± 34 in Con-H2O, and 92 ± 14 in Con-OβG. These were statistically different (P < 0.05, Fig. 1), with Ex-H2O and Ex-OβG mice having fewer tumors than Con-H2O mice (P < 0.001). Similarly, Con-OβG mice had fewer tumors than Con-H2O (P < 0.001). However, Ex-OβG was not different from Ex-H2O or Con-OβG, indicating that there were no additive effects of moderate exercise and oat β-glucan.

Peritoneal macrophage cytotoxicity. Peritoneal macrophage cytotoxicity, which reflects the ability of the macrophage to destroy tumor cells and/or limit tumor growth, was also measured against B16 melanoma cells. Peritoneal macrophage cytotoxicity was measured in macrophages taken within 30 min after the last exercise bout. Macrophage cytotoxicity was measured at four effector-to-target ratios, as seen in Fig. 2. There were significant main effects for both exercise (P < 0.05) and oat β-glucan (P < 0.05); however, there were no interactions. Peritoneal macrophages from Ex-H2O mice had significantly enhanced cytotoxicity compared with Con-H2O mice at two effector-to-target ratios (40:1 and 20:1; P < 0.05). Both OβG groups (Ex-OβG and Con-OβG) at 40:1, 20:1, and 10:1; P < 0.05) were also significantly increased over Con-H2O, but there were no additive effects of exercise and oat β-glucan.

DISCUSSION

Both moderate exercise and a healthy diet have been the focus of behavioral interventions designed to reduce the risk of cancer as well as other chronic diseases. Both exercise and oat β-glucan (as part of a Heart Healthy Diet) have well-documented benefits in the prevention of cardiovascular disease and diabetes, but much less is known about their specific role in cancer prevention. Much of the evidence is based on epidemiological and observational studies with little data from controlled experimental studies, and the mechanisms are largely unknown. This study used an established experimental tumor metastasis model found a decrease in the number of B16 melanoma cells. There were significant main effects for both exercise (P < 0.05) and oat β-glucan (P < 0.05); however, there were no interactions. Tumor counts were as follows: 95 ± 12.0 in Ex-H2O, 102 ± 12 in Ex-OβG, 189 ± 34 in Con-H2O, and 92 ± 14 in Con-OβG. These were statistically different (P < 0.05, Fig. 1), with Ex-H2O and Ex-OβG mice having fewer tumors than Con-H2O mice (P < 0.001). Similarly, Con-OβG mice had fewer tumors than Con-H2O (P < 0.001). However, Ex-OβG was not different from Ex-H2O or Con-OβG, indicating that there were no additive effects of moderate exercise and oat β-glucan.

Animal models of tumor development typically demonstrate that exercise inhibits tumorigenesis (3, 6, 15, 24, 30, 31, 33), although there are a few exceptions to this general finding (32, 39). A recent study in our laboratory using a similar experimental metastasis model found a decrease in the number of...
lung tumors after administration of B16 melanoma cells in response to a single bout of prolonged exercise to fatigue on a treadmill (6). However, this same study found no benefit of a single 30-min session of running. The beneficial effect of 6 days of moderate exercise in this study, along with related work by others (18, 22, 30, 31), suggests that intensity and duration as well as the timing of exercise onset are important variables in the protective effect of exercise.

A similar focus is being directed to the role of diet as a means for reducing the risk of cancer (13). Both epidemiological and animal experiments provide insight into the links between various dietary components in cancer prevention and treatment (13). Vegetables and fruits, dietary fiber, micronutrients, and phytochemicals have all been investigated for their potential role of the dietary components in cancer prevention and treatment (13). Vegetables and fruits, dietary fiber, micronutrients, and phytochemicals have all been investigated for their anti-tumor activity (13). Of particular recent interest has been the potential role of the dietary fiber β-glucan, which is an important component of a Heart Healthy Diet (16). β-Glucan is a polysaccharide that can be derived from the cell wall of yeast, fungi, and oats. Most of the anti-tumor data come from experiments involving the insoluble β-glucan from yeast and fungus in a variety of murine tumor models, including B16 melanoma, adenocarcinoma, mammary carcinoma, lymphocytic leukemia, and Lewis lung carcinoma, presumably acting via activation of both nonspecific and specific immune mechanisms (8, 27).

However, this is the first experiment on the effects of consuming soluble oat β-glucan in any cancer model. The protective effects shown here are in line with other similar studies. For example, Di Luzio et al. (8) reported that intravenous administration of β-glucan derived from yeast after subcutaneous injection of B16 melanoma cells resulted in a decrease in tumor growth and prolonged survival time in mice. Suzuki et al. (27) also reported an inhibitory effect of orally administered β-glucan derived from fungus on pulmonary metastases of Lewis lung carcinoma cells.

In the second series of experiments, we sought to explore potential mechanisms that may explain the benefits of moderate exercise and oat β-glucan on the reduction in tumor metastases resulting from the intravenous injection of B16 melanoma cells. Various host effector cells, including NK cells, and macrophages can kill a broad spectrum of tumor cells and do not require prior antigen priming to exert their cytotoxic effects. Both macrophages and NK cells are thought to play a role in limiting metastases by altering the early steps of implantation and growth of secondary foci (1, 11). Exercise-induced increases in the anti-tumor function of these cells are well documented (6, 17, 18, 20, 21, 23, 40, 41). For example, in addition to the increase in macrophage anti-tumor function shown here with 6 days of moderate-exercise training, we have also shown increases for up to 9 h after a single prolonged run to fatigue (6) and after 3 days of both moderate (30 min) and fatiguing exercise (40). Although the reduction in pulmonary metastasis cannot be directly related to the alveolar macrophage cytotoxicity, it is possible that rather long-lasting effects of macrophage activation might be responsible through a direct inhibition of infiltration and growth of the tumor cells as well as the release of antitumor and immunostimulatory cytokines (6, 38). Although NK cytotoxicity was not measured in this study, we have shown that this same exercise protocol can increase NK cytotoxicity to YAC lymphoma cells in mice (7).

The benefits of oat administration in this paper may also be attributed to activation of macrophages and NK cells (5, 26, 28, 34). These cells contain receptor sites specific for β-glucan on their cell surface membrane, such as complement receptor-3 and dectin-1 that, when combined with β-glucan, can upregulate macrophage and NK anti-tumor function (2, 5, 34). However, the mechanisms of stimulation are dependent on the route of administration (e.g., intravenous, intraperitoneal, or oral) and specific characteristics of the β-glucan, including the source (e.g., oats, yeast, fungi, etc.), solubility, molecular mass, degree of branching, and conformaion (ratio of 1→3 to 1→4 and 1→6 glucopyranosyl linkages) (9, 36). Orally administered β-glucan enhances both peritoneal and alveolar macrophage activity through an increase in acid phosphatase activity, phagocytosis, H2O2 production, and IL-1 production (25, 28).

This could result from ingestion of β-glucan by pinocytic M cells located in Peyer’s patches of the small intestine, causing a release of cytokines that are responsible for initiating an extensive cascade of systemic immune responses (14, 25, 26, 28). It is also possible for soluble oat β-glucan to be absorbed into the lymphatic and cardiovascular systems and thereby interact directly with circulating immune cells (16, 42). Therefore, it seems reasonable that the soluble oat β-glucan used in this experiment increased macrophage anti-tumor function, and perhaps other immune components, via direct activation via β-glucan-specific receptors on their cell surface (within the gut-associated lymphoreticular system and perhaps central circulation) and later through indirect activation via a cascade of systemic immune responses involving cytokines.

The results of this study suggest that short-term moderate-exercise training and consumption of the soluble fiber oat β-glucan can decrease the metastatic spread of injected B16 melanoma cells to the lungs in this experimental tumor metastasis model. The effects were associated with an increase in macrophage anti-tumor function against the same tumor cells in culture, which suggests a possible role for macrophages as mediators of these benefits. However, more research is required to determine the precise mechanisms of these effects.
and whether they can contribute to decreased risk of tumor metastasis in other more clinically relevant situations.

REFERENCES


