Effect of Voluntary Exercise on Azoxymethane-induced Colon Carcinogenesis in Male F344 Rats¹

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ABSTRACT

The effect of voluntary exercise on azoxymethane (AOM; CAS: 25843-45-2)-induced colon carcinogenesis was investigated in male F344 rats. Beginning at 5 wk of age, all animals were divided into two groups (sedentary and exercise) and fed AIN-76A semipurified diet ad libitum. At 7 wk of age, animals were given AOM s.c. at a dose level of 15 mg/kg of body weight, once weekly for 2 wk. Four days after the second dose of AOM, all animals in the exercise group were housed in individual wheel-cage units, and the animals in the sedentary group were housed in plastic cages. The experiment was terminated at 38 wk post-AOM treatment. Body weights of animals in the exercise and sedentary groups were comparable. The incidence (percentage of animals with tumors) and multiplicity (tumors/animal) of colon adenocarcinomas were significantly inhibited in the exercise group, but the incidence and multiplicity of colon adenomas were unaffected by the exercise. The incidence of small intestinal adenocarcinomas and liver foci was also inhibited in the exercise group.

INTRODUCTION

Epidemiological studies suggest that dietary factors, particularly high intake of total fat, saturated fat, and in some populations unsaturated fat, and a relative lack of dietary fiber may be of importance in the etiology of colon cancer in humans (1-4). The epidemiological evidence supporting total caloric intake as a risk factor for cancer of the colon emerged from a Canadian case-control study (5). Another case-control study in England suggested that high-energy diets rich in sugar and fat are associated with the development of colorectal cancer in humans (6). Animal model studies also confirmed the epidemiological evidence that colon tumor incidence is correlated with daily caloric intake (7).

If decreased energy intake or calorie restriction acts to inhibit colon carcinogenesis, then increased energy expenditure should exert a similar inhibitory effect. Support for the relationship between energy expenditure and colon cancer emerged from studies by Garabrant et al. (8) in Los Angeles, Vena et al. (9) in Buffalo, and Gerhardsson et al. (10) in Sweden, which suggest that the occupation-related physical activity may play a role in colon cancer etiology. The risk of colon cancer in males whose occupations were sedentary in nature far exceeded the risk of colon cancer in males whose jobs were physically oriented. The risk of colon cancer increased in a dose-response manner as the physical activity level decreased. However, none of the studies determined nutrient intake including total calories of the study subjects.

In laboratory animal models, it has been shown that forced exercise on a treadmill reduced 1,2-dimethylhydrazine-induced colon carcinogenesis in male F344 rats and that this effect is related to a concomitant reduction in body weight gain (11). In mammary carcinogenesis, forced exercise on a treadmill increased DMBA²-induced mammary tumor response in female Sprague-Dawley rats fed a high-fat diet, as compared to those not exercised (12), whereas voluntary exercise reduced methyl-nitrosourea-induced mammary tumorigenesis in female rats fed a high-fat diet (13). These results suggest that voluntary exercise has an inhibitory role in mammary carcinogenesis.

In this study, we investigated the effect of voluntary exercise on AOM-induced colon carcinogenesis in male F344 rats fed a semipurified diet.

MATERIALS AND METHODS

Animals, Diets, and Carcinogen. Weanling male inbred F344 rats were purchased from Charles River Breeding Laboratories (Kingston, NY); AOM (CAS: 25843-45-2) was from Ash-Stevens, Inc. (Detroit, MI); and all semipurified dietary ingredients were from Dyets, Inc. (Bethlehem, PA).

Animals received at weaning were quarantined for 10 days, randomly divided by weight into two experimental groups of 39 animals each, and designated as the sedentary group and the exercise group. Each group was subdivided into vehicle-treated (12 animals) and AOM-treated (27 animals) subgroups. Animals in the sedentary group were housed in plastic cages individually in a room maintained under controlled environmental conditions at 22°C, 50% humidity, and 12-h light-dark cycles. Rats in the exercise group were housed in the same room but in individual wheel-cage units, type H8002 (Lab Products, Inc., Maywood, NJ), consisting of a wire-bottomed stainless steel housing cage (10 x 6 x 5 inches) equipped with a running wheel (13.5-inch diameter and 4.5 inches wide) to which animals had free access. Attached to the running wheel was a mechanically coupled odometer which counted each turn, regardless of direction. One complete revolution of the wheel is equivalent to a distance of 3.53 ft; 1 mile equals 1496 revolutions. Each wheel cage unit had a food tray and water bottle attached. Animals had free access to food and water at all times. Starting at 5 wk of age, animals allotted to both experimental groups were fed a semipurified diet (AIN-76A). All diets were prepared in our laboratory once weekly and stored in a cold room.

Experimental Procedure. Beginning at 5 wk of age, all animals in each experimental group were fed the semipurified diet ad libitum. At 7 wk of age, animals intended for carcinogen treatment were administered s.c. with AOM for 2 wk with a dose level of 15 mg/kg of body weight, once weekly, whereas the animals intended for vehicle treatment were treated with an equal volume of normal saline. Before injection, AOM was dissolved in normal saline at a concentration of 10 mg/ml. Four days after the second injection of AOM or normal saline, all animals in the exercise groups were transferred to their respective exercise cages and maintained there until the termination of the experiment at 38 wk post-carcinogen administration. Body weights were recorded weekly until the animals attained 16 wk of age and then every 2 wk until termination of the experiment. At 10 wk after AOM injection, daily food consumption was measured for 5 days in 6 randomly selected animals from each group.

Both vehicle-treated and AOM-treated animals were sacrificed by CO₂ euthanasia as scheduled. At necropsy, all organs, including intestines, were removed, fixed in 10% formalin, and submitted to the Department of Pathology, Naylor Dana Institute, American Health Foundation, for histological examination. Gross excreta were also recorded for each experimental group.

The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; AOM, azoxymethane.

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Tumor Incidence. Table 2 summarizes the AOM-induced colon tumor incidence (percentage of animals with tumors) and colon tumor multiplicity (tumors/animal). The incidence and multiplicity of colon adenocarcinomas were significantly inhibited in the exercise group when compared to the sedentary group, but the incidence and multiplicity of colon adenomas were unaffected by the exercise.

Tumor incidence, other than colon, is shown in Table 3. All small intestinal tumors were located in the upper part of the duodenum. The incidence of small intestinal adenocarcinomas was significantly reduced in the exercise group. There were no differences in ear duct tumors between the experimental groups. Three types of liver hepatocellular foci, namely clear cell, eosinophilic cell, and basophilic cell foci, were found in all groups. The animals in the sedentary group developed significantly more eosinophilic and clear cell foci than did the rats in the exercise group. One animal in the sedentary group developed metastasis in the spleen. Two animals in the sedentary group showed liver nodules which are adenomas.

Histologically, adenocarcinomas of the colon and small intestine were well-differentiated frat malignant tumors, showing invasion across the line of the muscularis mucosa. Some of the colon tumors were poorly differentiated adenocarcinomas. Adenomas of the colon were benign with mild or moderate epithelial atypia. Liver tumors were adenomas and hepatocellular carcinomas. Tumors in the ear duct were squamous cell carcinomas.

DISCUSSION

Recent studies demonstrated that voluntary exercise similar to that performed in the current study effectively reduced mammary tumor yield and delay of time of appearance in a methylnitrosourea-induced tumor model (13), whereas forced treadmill running as the form of exercise increased DMBA-induced mammary tumor incidence in female rats (12). It can be concluded from these studies that intense, forced exercise, typical of treadmill running, may enhance mammary tumor development, while less intense exercise may act to inhibit tumor development. With respect to colon cancer, a recent report which appeared in abstract form, using the dimethylhydrazine model, indicated that forced treadmill running as the form of exercise caused a reduction in colon tumor incidence (11). The results of our present study suggest that voluntary (or less intensive) exercise induced fewer colon and small intestinal adenocarcinomas as well as produced a reduction in liver cancer. It is noteworthy that the laboratory animal model data are consistent with the epidemiological studies (8-10) that the occupation-related physical activity lowers the risk for the development of colon cancer in humans. In these studies, the degree of physical activity had no influence on rectal cancer. In the present study, exercise (or running) was voluntary but not...
forced, such as treadmill running, shock avoidance, and swimming, and food was provided *ad libitum*.

The pattern of wheel-running in the present study was consistent with that reported by others (15, 16), namely, an early peak followed by a gradual decline. However, there is a difference in quantity of wheel-running between male and female rats. For example, Cohen *et al.* (13) and Tokuyama and Okeuda (17) reported that female F344 and Wistar rats ran an average of 9,000 to 11,000 revolutions/day, which sum is greater than for male F344 rats in the present study. Our results also indicate a compensatory hyperphagia in exercise animals which has also been observed by others (18). Exercise animals consumed more calories than the sedentary animals.

The question arises as to how increased energy expenditure (exercise) may inhibit colon carcinogenesis. The mechanism of exercise-induced inhibition of colon carcinogenesis is remotely understood. In the present study, the protective effect of exercise did not correlate with the body weight gain, suggesting that this effect may be mediated by other than total-body mass. Also in mammary carcinogenesis, the antipromoting effect of increased energy expenditure did not correlate with either body weight, calorie intake, or the ratio of fat to lean-body mass (11). Most studies on the potential health benefits of exercise have focused on circulatory disorders and related changes in plasma lipoproteins (19, 20), longevity (21), β-endorphin levels (22), and enhanced immune functions (23). Garabrant *et al.* (8) proposed that physical activity stimulates colon peristalsis and decreases random, nonpropulsive segmentation activity. As a result, the contact between the colon mucosa and potential carcinogens in the colonic contents may be decreased by exercise, both because of the shortened transit time of the stool and because of the decrease in mixing that occurs during segmentation. It is unclear precisely how these changes may be related to colon carcinogenesis.

In summary, our results suggest that voluntary exercise inhibits colon carcinogenesis in the animal model. These results are in line with the epidemiological evidence.

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