Reduction in the incidence of radiation-induced tumors in rats after restriction of food intake

(spontaneous rat tumors/caloric restriction/γ-irradiation)

LUDWIG GROSS and YOLANDE DREYFUSS

Cancer Research Unit, Veterans Administration Medical Center, Bronx, NY 10468

Contributed by Ludwik Gross, August 9, 1984

ABSTRACT In our inbred colony of Sprague–Dawley rats, fractionated total-body x-ray irradiation (150 rads five times at weekly intervals; 1 rad = 0.01 gray) increased the incidence of tumors from 22% to 93% in females and from 5% to 59% in males (Gross, L. & Dreyfuss, Y. (1979) Proc. Natl. Acad. Sci. USA 76, 5910–5913). In experiments reported here, we investigated the influence of reduced food intake on the incidence of radiation-induced tumors in Sprague–Dawley rats. All rats in both groups received fractionated total-body x-ray irradiation similar to that specified above. Among the irradiated rats on full (ad lib) diet (five to six pellets of Purina Rodent Lab Chow per day, each) were 14 females and 9 males, and all (100%) developed tumors (or leukemia) at an average age of 13.7 months in females and 13.4 months in males. Among the litter mates on restricted diet (two pellets of Purina Rodent Lab Chow per day, each) only 9 out of 29 females (31%) developed tumors at an average age of 18.2 months and 1 out of 15 males (6.7%) developed a tumor at 9 months of age. In the full diet group, the majority of tumors developing in females were benign. Among the 9 irradiated males, 1 developed leukemia and the remaining 8 developed tumors; 7 of them were examined, 6 were found malignant, and 1 was benign. In the restricted diet group, of the 9 tumors that developed in females, 4 were malignant and 5 were benign. The tumor that developed in 1 male was a sarcoma.

It has been known since the early work of Sivertsen and Hastings (1), Tannenbaum (2, 3), and their colleagues that the incidence of mammary carcinomas in certain inbred strains of mice could be substantially reduced by restriction of caloric food intake. Subsequently, Saxton and his colleagues (4) observed that the development of spontaneous leukemia in the high-leukemic AK strain of mice could be considerably reduced by restriction of caloric food intake.

It appeared of interest to determine whether a similar reduction in the incidence of tumors, following restriction of food intake, would occur also in another species of animals. The principal difficulty was to find a suitable animal model with a relatively high incidence of tumors.

The incidence of spontaneous tumors in rats is significant, but relatively low. In our colony of inbred Sprague–Dawley rats we observed an average incidence of tumors of 22% in females and 5% in males, developing after an average latency of about 18 months (5). The majority of the tumors in females originate in the mammary glands. Only about one-third of these tumors are malignant. Some tumors, classified as "benign" on the basis of cell morphology, grow progressively and may reach very large size; frequently, rats develop multiple tumors, and malignant and nonmalignant neoplasms may occur in the same animals. However, we observed that the incidence of these tumors could be considerably increased after total-body x-ray irradiation, from 22% to 93% in females and from 5% to 59% in males, with simultaneous shortening of the average latency period from 18 to <12 months. The incidence of malignant tumors was almost twice as high in the irradiated rats as compared with nonirradiated animals of the same strain (5). The incidence of leukemia was not significantly increased by total-body x-irradiation, whereas in previous studies (6–8), as well as in our own earlier experiments carried out on mice (9), a similar total-body x-irradiation increased dramatically the incidence of leukemia and lymphomas in mice of several (but not all) inbred lines of that species (7, 9). Radiation-induced leukemia in mice was subsequently found to be caused by a virus (10, 11) that could be passed serially in newborn mice, inducing leukemia and lymphomas in that species (12).

Accordingly, it appeared that we have in our hands an excellent animal model for the study of the possible influence of food restriction on the incidence of radiation-induced tumors. This was the subject of the current study.

MATERIALS AND METHODS

Animals. From a nucleus of random-bred Sprague–Dawley rats received in June 1960 from the Animal Production Unit, National Institutes of Health, a colony of rats has been raised in our laboratory by brother-to-sister mating. No animals from outside sources have been added; only those bred in our laboratory have been used in this study.

Technique of Total-Body X-Irradiation. Young adult Sprague–Dawley rats received at the Radiotherapy Department of this Medical Center five consecutive total-body x-irradiations of 150 rads (1 rad = 0.01 gray) each, at weekly intervals. The animals were 3–4 weeks old when they received the first irradiation. The animals were placed in small plastic compartments, 10 cm wide and 15 cm long, each holding one rat; up to six rats in six compartments were irradiated at one time. The technical factors were as follows: Picker Cobalt-60 teletherapy unit, 80-cm source-to-surface distance, 81.5-cm source-to-midplane of rat distance, portal size of 33 × 30 cm for six compartments, dose rate of 100 rads per minute.

Food Intake. The rats were fed with Purina Rodent Lab Chow pellets. These pellets are rectangular: 2.5 to 3 cm long, 1.5 cm wide, and 1 cm thick; the approximate weight of each pellet varies from 4.7 to 5.0 g. In the group on full diet, when allowed to eat ad lib, each of the rats consumed about five to six, occasionally seven, Purina Rodent Lab Chow pellets per day. The rats on restricted diet received only two pellets per day.

RESULTS

A total of 51 Sprague–Dawley females and 29 males, all littermates, was divided into three groups. In the first group, 14 females and 9 males received x-irradiation only; they were allowed to eat as much as they desired and consumed an average of five to six pellets of Purina Rodent Lab Chow per day, each; males ate slightly more than females. In the
second group, 29 females and 15 males, sisters and brothers of those in the first group, received the same total-body x-irradiation; however, they were placed on a restricted diet of two pellets of lab chow per day, each. The third group, consisting of 8 females and 5 males, received a restricted diet of two pellets of lab chow per day and were not subjected to x-irradiation.

In the first group, all 14 irradiated females developed tumors at ages varying from 8.5 to 18 months (average, 13.7 months). Eleven tumors were examined microscopically: 10 were fibroadenomas and 1 was a fibrosarcoma. Of the 9 irradiated males, 1 developed myelogenous leukemia at 8.5 months, and the remaining 8 males developed tumors at ages ranging from 8.5 to 22 months (average 13.4 months). Seven of these tumors were examined microscopically: 5 were sarcomas, 1 was a carcinoma, and 1 a fibroadenoma.

In the second group, among the 29 irradiated females on restricted diet, sisters of those in the preceding group, only 9 developed tumors at ages ranging from 8 to 26 months (average 18.2 months). Three of these tumors were carcinomas, 1 was a fibrosarcoma, and the remaining 5 were benign—either fibroadenomas or fibroangiomas. Among the 15 irradiated males on restricted diet, brothers of those in the first group, only 1 developed a subcutaneous sarcoma at the age of 9 months.

In the third group, eight females and five males were fed a restricted diet, but did not receive any radiation. None of these animals developed tumors or leukemia during the period of observation of 21.5 to 24 months (Table 1).

In our previous study, 22% of untreated Sprague–Dawley females and 5% of untreated males developed spontaneous tumors at an average age of 18 months and 17 months, respectively; in addition, a few among them developed leukemia and lymphomas (5).

DISCUSSION
We have investigated the effect of reduced food intake on the incidence of radiation-induced tumors in Sprague–Dawley rats. Preliminary results thus far obtained suggest that the incidence of radiation-induced tumors in rats could be substantially reduced by placing these animals on a restricted diet. In our present study, here reported, all 14 females and 9 males in the irradiated group on full diet developed tumors (100%), as compared with only 9 of 29 females (31%), and 1 of 15 males (6.7%), in the irradiated group on restricted diet (Table 1). The rats on restricted diet (two Purina Rodent Lab Chow pellets per day, each) remained in relatively good health, but their weight was reduced by almost half, as compared with the average weight of animals receiving a full—i.e., ad lib—diet (five to six, occasionally seven, pellets per day, each).

The significant reduction in the incidence of tumors developing in rats on restricted diet, was essentially similar to results of earlier experiments, describing a similar reduction in the incidence of either mammary carcinomas (1–3) or leukemia (4) in mice of certain inbred lines maintained on restricted diets.

All of our animals in the irradiated group on full diet developed tumors when about 1 year old, whereas the majority of those on restricted diet died without tumors at an average age of 18.8 months for females, and 14.7 months for males, or are still alive at about 2 years of age (Table 1). Whether the restriction of diet actually prevents the development of tumors or only delays their appearance remains to be determined. Not many untreated rats in our laboratory live longer than 27 or 28 months; only relatively very few reach or pass 2.5 years of age. This appears to be also the experience in other laboratories (13).

Among the additional points of interest observed in our present study were the observations that restriction of food intake resulted in the reduction of a variety of not only malignant but also benign tumors and, furthermore, that the oncogenic effect of x-irradiation on rats could be influenced by restriction of food intake.

The mechanism of the prevention of, or a significant delay in, the development of tumors in rats on restricted diet is still obscure. In similar observations made earlier on mice, among several other interpretations it was possible to theorize that the reduction in the incidence of tumors or leukemia in animals on restricted food intake, could have been caused by prevention of, or a significant delay in, the activation of oncogenic viruses, carried by such mice. Both mammary carcinoma and leukemia are known to be caused by viruses in that species.

Although we suspect that radiation-induced tumors, as well as tumors developing in rats spontaneously, are caused by oncogenic viruses, we have no experimental evidence at the present time to substantiate such an assumption. Tumors induced in rats by total-body x-irradiation, as well as those developing in rats spontaneously, could not be transmitted in our laboratory by cell-free extracts; furthermore, no virus particles could be found, thus far, by electron microscopy in any of the tumor specimens examined (5).

Other explanations of the mechanism or mechanisms preventing or delaying the development of tumors in animals on restricted diet must also be taken under consideration. They may apply to rats as well as to mice.

Restriction of food intake may prevent the initial development of tumors or leukemia, but it will not significantly influence the growth or progress of established tumors in mice (14).
We appreciate very much the cooperation of Dr. Bernard Roswit, former Chief, and that of Dr. Julian Tenner, Assistant Chief of the Radiotherapy Service, as well as the assistance of Mr. Cyprian Reid, former staff physicist, and the technicians of the Radiotherapy Service at this Medical Center. We also thank Dr. T. E. Ehrenreich, our consultant in pathology, for his evaluation and comments on the microscopic sections, and Mrs. Lorraine Moore Limbert for the preparation of microscopic slides in pathological studies of rat tumors. This study was supported by the Veterans Administration Research Service, the American Cancer Society Research Development Program Grant RD-183, and a grant from the Cancer Research Institute, New York.
