Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6795–6797, September 1990
Medical Sciences

Prevention of spontaneous and radiation-induced tumors in rats by reduction of food intake

(γ-irradiation/caloric restriction)

LUDWIK GROSS*† AND YOLANDE DREYFUSS*

*Cancer Research Unit, Veterans Affairs Medical Center, Bronx, NY 10468; and †The Cancer Center of the Mount Sinai School of Medicine, City University of New York, New York, NY 10029

Contributed by Ludwik Gross, May 31, 1990

ABSTRACT **In our previous studies carried out on inbred Sprague–Dawley rats, we reported a striking increase in the incidence of tumors following total-body γ-irradiation [150 rads (1.5 Gy) five times at weekly intervals]. Subsequently, we observed that two or three irradiations, and to a lesser extent even a single irradiation, were sufficient to induce an impressive increase in the incidence of tumors, particularly in females. A significant reduction of the incidence of radiation-induced tumors resulted when the rats were placed on calorically restricted diet. In experiments reported here, we increased slightly the amount of food given to animals on restricted diet. In the new study, among 102 irradiated females on full diet, 91 (89%) developed tumors, as compared with 29 out of 128 female rats (23%) also irradiated but maintained on restricted diet and 43 out of 89 (48%) untreated control females. None of 77 nonirradiated females on restricted diet developed tumors. Among 65 irradiated male rats, 29 (45%) developed tumors, as compared with 5 out of 74 (7%) rats also irradiated but maintained on restricted diet. Of the 49 males in the nonirradiated groups, 2 (4%) developed tumors. There was a significant weight reduction in both females and males maintained on restricted diet; animals on restricted diet lived longer than those on full diet.**

We have demonstrated in our previous studies (1) that the incidence of tumors in an inbred subline of Sprague–Dawley rats was considerably increased by fractionated total-body γ-irradiation. In the initial studies, we applied five consecutive irradiations at weekly intervals. In subsequent studies, however, we have learned that two or three irradiations (2), and to a lesser extent even a single irradiation, were sufficient to induce a high incidence of tumors, particularly in females. Sprague–Dawley rats, like those of several other strains, are prone to the spontaneous development of tumors, mostly benign and mostly in females (1, 2). To have background information based on a sufficiently large number of untreated animals, we have reviewed our data on the incidence of tumors or leukemia in a large number of untreated rats of our Sprague–Dawley inbred subline (Table 1).

In our previous studies (3, 4), we observed a striking reduction of the incidence of radiation-induced tumors in Sprague–Dawley rats after restriction of food intake. In the present study, we report additional experiments on Sprague–Dawley rats that were submitted to three or five consecutive γ-irradiations and received a slightly increased diet; nevertheless, the effect in these experiments—a significant reduction in the incidence of tumors—was striking (Table 2). We also reviewed in this report the weight of rats maintained on reduced food intake (Table 3) and the effect of food restriction on their longevity (Table 4).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Animals. From a nucleus of random-bred Sprague–Dawley rats received in June 1960 from the Animal Production Unit of the 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 γ-Irradiation. Young adult Sprague–Dawley rats received at the Radiotherapy Department of this Medical Center three or five consecutive total-body irradiations of 150 rads (1.5 Gy) 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, and a dose rate of 100 rads per min.

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 rat consumed about five to six, occasionally seven, Purina Rodent Lab Chow pellets per day. The rats on restricted diet received two pellets daily cut to weigh 3.5 g each in group I; two and three pellets on alternate days in group II; and 2 pellets daily in groups III, IV, and V, except that once a week, on Thursdays, they received three pellets each, instead of two.

RESULTS

Table 1 summarizes our data from 1966 to 1986, referring to the incidence of spontaneously developing tumors or leukemia in our subline of Sprague–Dawley rats bred in our laboratory by brother-to-sister mating. Among 313 females, 82 (26%) developed tumors, most frequently of the mammary glands, of which the great majority were benign (2), and only 5 developed leukemia. Among the 316 males, only 10 (3%) developed tumors, most frequently sarcomas, and 4 developed leukemia.

In the present study, after three to five fractionated total-body γ-irradiations, the incidence of tumors in Sprague–Dawley rats increased considerably in both males and females. Table 2 summarizes the effects of total-body γ-irradiations and the inhibiting effect of the reduction of food intake on the incidence of either radiation-induced or spontaneously developing tumors. In the irradiated group of 102 female rats, 91 (89%) developed tumors, as compared with 29 out of 128 (23%) irradiated females maintained on restricted diet and 43 out of 89 (48%) untreated controls; none of the 77 nonirradiated females on restricted diet developed tumors.

Table 1. Incidence of spontaneous tumors and leukemia in Sprague–Dawley rats

			Rats developing tumors					Rats developing leukemia					Rats that died negative			
					Age at tumor development, mo					Age at leukemia development, mo				Age at death, mo		
Exp. series	Sex	No. rats obs.	No.	%	Min	Max	Avg	No.	%	Min	Max	Avg	No.	Min	Max	Avg
1	F	133	29	22	9	28	18	5	4	5	19	15.8	99	6	28	17
	M	141	7	5	9	26	17	2	1	19	28	23.5	132	6	31	17
2	F	78	23	29	14	28	20	0	0	—	—	—	55	7	28	16
	M	75	1	1	14	14	14	0	0	—	—	—	74	6	29	17
3	F	62	17	27	10	24	18	0	0	—	—	—	45	8	26	18
	M	60	2	3	17	20	18	2	3	17.5	18	17.7	56	9	25	16
4	F	20	8	40	15	22	19	0	0	—	—	—	12	7	23	17
	M	25	0	0	—	—	—	0	0	—	—	—	25	9	27	18
5	F	20	5	25	13	22	19	0	0	—	—	—	15	6	23	16
	M	15	0	0	—	—	—	0	0	—	—	—	15	11	27	19
Total	F	313	82	26.2				5	1.6				226			
	M	316	10	3.1				4	1.2				302			

Series 1 experiments were published in 1979 (1); series 2 studies, in 1980–1982; series 3 studies, in 1982–1984; series 4 studies, in October 1984 to July 1985; series 5 studies, in August 1985 to August 1986. Most of the females were from our breeding colony and had litters prior to the development of tumors. F, female, M, male, obs., observed; TD, tumor development; Min, minimum; Max, maximum; Avg, average; mo, month(s).

Table 2. Reduction of the incidence of spontaneous and radiation-induced tumors in Sprague–Dawley rats following restriction of food intake

		Group I					Group II					Group III					Group IV					Group V					Total				
			Rats dev. tumors					Rats dev. tumors					Rats dev. tumors					Rats dev. tumor					Rats dev. tumor					Rats dev. tumor			
Treatment	Sex	No. rats obs.	No.	%	Age at TD, mo	No. rats dev. Leuk*	No. rats obs.	No.	%	Age at TD, mo.	No. rats dev. Leuk*	No. rats obs.	No.	%	Age at TD, mo	No. rats dev. Leuk*	No. rats obs.	No.	%	Age at TD mo	No. rats dev. Leuk*	No. rats obs.	No.	%	Age at TD, mo	No. rats dev. Leuk*	No. rats obs.	No.	%	Age at TD, mo	No. rats dev. Leuk*
γ-Rays	F	14	14	100	14	0	31	24	77	12	0	34	31	91	12	1	23	22†	96	12	1	0	—	—	—	—	102	91	89	—	2
	M	9	9	100	13	0	17	1	6	13	0	19	10	53	15	0	20	9	45	15	1	0	—	—	—	—	65	29	45	—	1
γ-Rays/diet	F	29	9	31	18	0	34	13	38	15	0	40	6	15	16	0	25	1	4	10	0	0	—	—	—	—	128	29	23	—	0
	M	15	1	7	9	0	19	1	5	16	0	24	3	12	17	0	16	0	—	—	0	0	—	—	—	—	74	5	7	—	0
Diet only	F	8	0	—	—	0	26	0	—	—	0	0	—	—	—	—	13	0	—	—	0	30	0	—	—	1	77	0	—	—	1
	M	5	0	—	—	0	17	2	12	28	0	0	—	—	—	—	3	0	—	—	0	24	0	—	—	1	49	2	4	—	1
Controls	F	0	—	—	—	—	25	9	36	20	1	19	12	63	17	0	19	9	47	15	1	26	13	50	15	2	89	43	48	—	4
	M	0	—	—	—	—	14	0	—	—	0	7	0	—	—	—	16	0	—	—	1	23	0	—	—	1	60	0	—	—	2

Groups I, II, and III received five irradiations each at weekly intervals. Group IV received only three irradiations, and group V received none. Animals on diet in group I received two Purina Lab Chow pellets every day. Those in Group II received two and three pellets on alternate days. Rats on diet in Groups III, IV, and V received two pellets daily and three once a week on Thursday. F, females; M, males; Leuk, leukemia; TD, tumor development; dev., developing; obs., observed; mo, months.

*Age of leukemia development was 5.5–18 months; the average age was 11.5 months.

†One rat developed a tumor and leukemia.

Table 3. Average weight of Sprague–Dawley rats on full and on restricted diet

		Average weight of rats with time, g					
		Group II*			Group V†		
Treatment	Sex	3 mo	6 mo	12 mo	3 mo	6 mo	12 mo
γ-Rays	F	236	251	261	—	—	—
	M	325	367	418	—	—	—
γ-Rays/diet	F	176	195	180	—	—	—
	M	200	216	150	—	—	—
Diet only	F	190	193	189	162	174	184
	M	211	216	208	169	181	187
Controls	F	340	278	315	270	302	312
	M	331	384	415	426	521	520

All animals referred to in Table 2 were weighed each month (mo); the weights were tabulated and compared. Control rats and those treated with γ-irradiation only were on full diet. mo, Months.
*Designation of groups is the same as in Table 2. Rats in group II received five consecutive total-body γ-irradiations. Animals on diet in that group received two and three Lab Chow pellets (3.5 g each) on alternate days.
†Rats on diet in group V received two pellets daily except Thursdays, when they received three pellets.

Among the irradiated group of 65 male rats, 29 developed tumors (45%), as compared with 5 out of 74 also irradiated but maintained on restricted diet (7%). None of the 60 untreated control male rats and 2 among the 49 nonirradiated males on restricted diet (4%) developed tumors. A few males and females, particularly in the control group, developed leukemia (Table 2). It is evident from the results summarized in group V, that restriction of diet alone entirely prevented the development of tumors in 30 females, as compared with 13 developing tumors in the control group of 26 littermates (50%) receiving as much food as they desired.

The rats on restricted diet lost weight (Table 3) but were in good health, physically active (running around in their cages, particularly before feeding time), and living longer than the control animals maintained on a full diet. Most of the animals on reduced diet survived their brothers and sisters in other experimental groups by 2–10 months (Table 4).

Table 4. Average survival time in months of rats on full and restricted diets

Treatment	Sex	Group I	Group II	Group III	Group V
γ-Rays	F	—	13	11	—
	M	—	14	14	—
γ-Rays/diet	F	19	19	20	—
	M	15	21	16	—
Diet only	F	19	24	—	26
	M	13	20	—	19
Controls	F	—	14	21	19
	M	—	14	19	17

Groups were the same as listed in Tables 2 and 3.

DISCUSSION

Experiments reported in this study confirm and extend our previous data. It is apparent that total-body γ-irradiation increases considerably the incidence of tumors developing in rats and that the carcinogenic potential of ionizing radiation and the incidence of spontaneously developing tumors can be significantly inhibited by reduction of food intake. We also observed that restriction of food intake had a beneficial effect on the longevity of our animals.

The etiology of either spontaneous or radiation-induced tumors in rats remains to be clarified. Our attempts (*i*) to detect virus particles be electron microscopy in tumors or lymphomas that either developed spontaneously or were induced by γ-irradiation or (*ii*) to transmit such tumors or lymphomas from rats to rats by cell-free extracts have not succeeded thus far. We are tempted to theorize that tumors or lymphomas developing in rats spontaneously or induced by irradiation are caused by latent viral agents that are integrated into the cell genome and are cell-associated—i.e., not separable from the tumor or leukemic cells by conventional methods thus far used.

This manuscript is dedicated to the memory of Dorothy Nelson Gross, wife and helpful companion of the senior author of this paper. This study was supported by the Veterans Affairs Medical Research Service and the Philanthropic Fund of the Department of Neoplastic Diseases (Dr. James F. Holland, Professor and Chairman), Mount Sinai School of Medicine, City University of New York, and by a grant from the Cancer Research Institute Inc. (New York).

1. Gross, L. & Dreyfuss, Y. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5910–5913.
2. Gross, L., Dreyfuss, Y. & Faraggiana, T. (1988) *Cancer Res.* **48,** 2451–2453.
3. Gross, L. & Dreyfuss, Y. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7596–7598.
4. Gross, L. (1988) *Cancer* **62,** 1463–1465.