

Synergistic Effect of Radiation on N-2-Fluorenylacetamide-induced Hepatocarcinogenesis in Male ACI/N Rats

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The effect of radiation on chemical hepatocarcinogenesis was examined in 3 groups of male ACI/N rats. In Group I, 21 rats received dietary administration of N-2-fluorenylacetamide (FAA) (0.02%) for 16 weeks. Six of the rats were killed at the cessation of FAA exposure. The remaining rats were then given the basal diet until termination (32 weeks). In Group II, 16 rats were given FAA for 16 weeks. The animals were then given radiation (whole body; 3 Gy) and kept on the diet for the subsequent 16 weeks. Thirteen rats of Group III were kept on the basal diet throughout the experiment. They received radiation for 16 weeks after the start of the experiment. Liver tumors were obtained in Groups I and II. The multiplicity of the neoplastic nodules or hepatocellular carcinomas of Group II (6.5 ± 2.5 or 1.4 ± 0.9) was significantly greater than that of Group I (2.9 ± 1.7 or 0.3 ± 0.4 , respectively) ($P < 0.001$). Furthermore, the incidence of hepatocellular carcinoma of Group II (13/16) was also significantly higher than that of Group I (4/15) ($P < 0.003$). The results clearly indicate a synergistic effect of radiation with FAA on the hepatocarcinogenesis. The effect of radiation in this rat model appeared to be on the early progression of the carcinogenesis.

Key words: Synergism — X-irradiation — Hepatocarcinogen — Rat — Progression

X-Irradiation has been reported to elevate cancer incidence in various organs of humans.¹ However, the oncogenicity of radiation itself in the human liver is obscure. In fact, no increase in the incidence of liver cancer has been reported among the atomic bomb survivors.^{2,3} In experimental studies, X-irradiation has been shown to induce cancers in different organs of various species of experimental animals.^{1,4} However, X-irradiation itself is regarded not to be carcinogenic in the liver of rats,^{4,5} although it was shown to induce hepatic neoplasms in mice, especially when perinatal animals were used.^{6,7} Recent studies on the potential of X-rays using preneoplastic lesions of rat liver are in agreement with these reports.^{8,9} For instance, Kitagawa *et al.*⁸ showed that although X-rays were capable of inducing early lesions in rats, the capability was very low compared with that of a chemical hepatocarcinogen. However, humans are exposed to radiation together with hazardous chemicals in the environment, and radiation and a number of chemotherapeutic agents, particularly alkylating agents, have been used together for therapeutic purposes. Although synergistic effects of radiation with chemical carcinogens have not been widely investigated,¹⁰ there is some evidence for synergism between radiation and carcinogens.^{11,12} In these studies, radiation was mainly applied in the initiation phase, and little information is available on the synergistic effect of

radiation with hepatocarcinogens. We were interested in the supplemental effect of X-irradiation on chemical hepatocarcinogenesis, and therefore we performed whole-body irradiation when rats had developed preneoplastic hepatocellular foci^{13,14} together with a few benign liver cell neoplasms.

Recent work on the multistage nature of neoplastic development has focused on progression or agents acting as progressors. Since radiation lacks definite hepatocarcinogenicity in rats in spite of its clear genetic effects, radiation might act as a progressor of hepatocarcinogenesis in rats.

MATERIALS AND METHODS

Three groups of male rats of ACI/N, a strain which has been maintained as an inbred line at our laboratory,¹⁵ 1.5 months old at the commencement, were used in the study. Group I. Twenty-one rats were fed a basal diet, CE-2 (CLEA Japan Inc., Tokyo) mixed with N-2-fluorenylacetamide (FAA) (Nakarai Chem. Co., Kyoto) at a concentration of 0.02% for 16 weeks. Six of them were killed at the termination of administration of the hepatocarcinogen (Group I-a). This subgroup was set up to confirm the state of FAA-induced hepatocarcinogenesis at the time of discontinuation of the carcinogen exposure when X-irradiation was applied. The remaining 15 rats were then fed the basal diet without carcinogen for the subsequent 16 weeks (Group I-b). Group II.

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Sixteen rats were given FAA for 16 weeks as done in Group I. They were then given whole-body X-irradiation of 3 Gy (200 kVp; 20 mA; half-value layer, 1.2 mm Cu; 0.27 Gy/min), and fed the basal diet for a further 16 weeks. Group III. Thirteen rats received the basal diet throughout the experiment (32 weeks). They were given irradiation 16 weeks after the start of the experiment. Diet and water were given *ad libitum*. The animals were autopsied after being killed by decapitation. At autopsy, the livers were weighed, and each lobe was carefully observed, fixed in 10% buffered formalin and embedded in paraffin. The liver tissues were observed micro-

scopically after being stained with hematoxylin and eosin. The area of the liver sections was calculated with a Digigrammer Model-G (Mutoh Inc., Tokyo) and the data on altered foci were expressed as number per cm².

RESULTS

All of the 6 rats killed at the termination of 16 weeks' feeding of FAA-containing diet (Group I-a) had altered hepatocellular foci.^{13,14)} Averaged incidence of the lesions was 83.9±18.8 per cm². Among these foci, the eosinophilic type was most frequent (93.5±1.2%). Other types

Table I. Incidence and Type of Altered Hepatocellular Foci of Each Group

Group (Treatment)	No. of rats examined	Incidence of the lesions/cm ² of liver ^{a)}	Types of lesions (%)		
			Eosinophilic	Clear cell	Basophilic
I					
(FAA 16 weeks)					
a	6 ^{b)}	83.9±18.8	93.5±1.2	3.6±0.8	2.9±0.7
b	15	68.2±10.3	89.8±3.9	9.4±3.4	0.8±1.8
II					
(FAA 16 weeks + radiation)	16	105.5±35.3	89.3±4.9	7.4±4.5	3.3 ^{c)} ±2.7
III					
(Radiation alone)	13	0	—	—	—

The data were obtained from the animals at the end of the experiment except for Group I-a in which rats were killed at the termination of FAA exposure.

a) Area of neoplasms is excluded.

b) Two rats had solitary hepatocellular neoplastic nodules.

c) Significantly larger than the corresponding number of Group I (*P*<0.05).

Table II. Incidence and Multiplicity of Liver Neoplasms of Each Group at the End of the Experiment

Group (Treatment)	No. of rats examined	Neoplastic nodule		Cholangiocellular adenoma		Hepatocellular carcinoma	
		No. of rats with	No. of tumors/rat	No. of rats with	No. of tumors/rat	No. of rats with	No. of tumors/rat
I							
(FAA 16 weeks)	15	14 (93)	2.9±1.7	0 (0)	0	4 (27)	0.3±0.4
II							
(FAA 16 weeks + radiation)	16	16 (100)	6.5±2.5 ^{a)}	1 (6)	0.06	13 ^{b)} (81)	1.4±0.9 ^{a)}
III							
(Radiation alone)	13	0 (0)	0	9 (0)	0	0 (0)	0

(): %.

a) Significantly larger than the corresponding number of Group I (*P*<0.001).

b) Significantly larger than the corresponding number of Group I (*P*<0.003).

of foci were seen in the following proportions: clear cell type, $3.6 \pm 0.8\%$; basophilic cell type, $2.9 \pm 0.7\%$ (Table I). Out of 6 rats of this group, 2 rats had neoplastic nodules in the livers (Table I). They were solitary neoplasms. At the termination of the experiment (16 weeks after the cessation of carcinogen exposure), 14 out of 15 rats of Group I had neoplastic nodules in the liver. The number of neoplastic nodules was 44 in total. Besides these benign neoplasms, a total of 4 hepatocellular carcinomas were seen in 4 rats of this group. In Group II, a total of 105 neoplastic nodules of the liver were seen in 16 rats. In this group, 23 hepatocellular carcinomas were observed in 13 out of 16 rats. Furthermore, one rat of the group developed a cholangiocellular adenoma (Table II). In Group III, no altered hepatocellular focus was confirmed in any rat. No liver neoplasm was seen in this group either (Table I). Statistically, the incidence of altered foci of basophilic type in Group II was significantly greater than that of Group I-b ($P < 0.05$) (Table I). The incidence of hepatocellular carcinoma of Group II (FAA + X-ray) was higher than that of Group I (FAA alone) ($P < 0.003$). Furthermore, the multiplicity of neoplastic nodules and hepatocellular carcinomas of Group II was larger than the corresponding value of Group I ($P < 0.001$).

DISCUSSION

Altered hepatocellular foci have generally been regarded as possible precursor lesions for neoplastic nodules or hepatocellular carcinomas,^{13,14} and are used as a critical parameter for quantitative analysis of initiators or modulators in experimental hepatocarcinogenesis. Dietary administration of FAA at the concentration and for the duration used in the present experiment induces various types of altered hepatocellular foci without malignant liver neoplasms in ACI/N rats within a short period.¹⁶ In this study, altered hepatocellular foci were not seen in rats of Group III given R alone. The results are basically in agreement with previous evidence showing weak initiating activity of X-irradiation in rats.⁹ Nevertheless, Kitagawa *et al.*⁸ reported induction of a relatively high incidence of altered hepatocellular foci by X-irradiation (4 Gy) in Wistar-Ms rats which received radiation at 8 or 22 days of age and were killed at 22 weeks of age. Thus, the discrepancy between that report and the present result may be attributed to the age difference of the rats at radiation treatment and to the difference of rat strains. In this experiment, the incidence of hepatocellular carcinoma of Group II was much higher than that of Group I. Furthermore, the multiplicity of neoplastic nodules or hepatocellular carcinomas of Group II was also much greater than that of Group I. It appears that the single exposure to X-irradiation promoted the transi-

tion of altered hepatocellular foci to neoplastic nodules or to hepatocellular carcinomas and the transition of the nodules to the carcinomas. These results clearly indicate an enhancing effect of radiation on FAA-induced hepatocarcinogenesis in rats, demonstrating an apparent synergism of radiation and the chemical carcinogen. Many studies have demonstrated possible initiating effects of radiation for induction of neoplasms in rodents,^{1,6,8,9} but only a few have proved synergistic effects of radiation with chemical carcinogenesis.^{11,12} Lurie¹¹ reported an interaction between 7,12-dimethylbenz[*a*]anthracene and X-irradiation in hamster cheek pouch carcinogenesis, and Sharp and Crouse¹² showed a synergism between radiation and 1,2-dimethylhydrazine in the induction of colonic tumors in rats. Meanwhile, Peraino *et al.*¹⁷ demonstrated a synergistic effect of γ -irradiation with diethylnitrosamine at the precancerous stage of hepatocarcinogenesis. A similar study has been done by Enomoto *et al.*,¹⁸ who employed X-rays in the Solt-Farber model where FAA was used as a selection procedure. In almost all of these studies, radiation was used as a coiniciating agent. In the present study, radiation was applied when chemically induced precancerous lesions had already developed, to examine the supplemental effect of radiation in carcinogenesis. The results of this study suggest a promoting effect of radiation in the two-stage carcinogenesis. However, X-rays are considered to cause genetic damage to the cells in altered hepatocellular foci and benign liver neoplasms. Changes in the genomes of these cells caused by a single exposure to radiation seem to generate more benign or malignant hepatocellular tumors in rats. Furthermore, the supplement of radiation enriched basophilic altered foci in the present experiment. Our previous study demonstrated that this type of focus had a more characteristic heterogeneity than other types.¹⁶ Thus, it may be appropriate to assume that radiation acts as a tumor progressor rather than a tumor promoter. Agents acting as progressors have not yet been definitely characterized. Current proposals for the "progressors" are that the agents should be capable of inducing genetic changes and thus should exhibit some degree of clastogenic activity.^{19,20} Examples of such agents are free radical generators such as those capable of inducing the stage of progression in experimental epidermal carcinogenesis.²¹ It is known that production of modifications in enzymatic activities of rat hepatocytes leads to increased lipid peroxidation by γ -irradiation.²² Single exposure to a relatively low dose of X-rays as used here, thus, could be useful for investigation of tumor progression in the model of hepatocarcinogenesis.

The application of combined modalities for treatment of malignancies, unfortunately, has been suggested to lead to an increased risk of second malignancies.²³ It is

quite probable that humans having some preneoplastic lesions or other premalignant lesions, are exposed to radiation. Accordingly, experimental results such as

those presented here could have implications for the prevention of human cancer.

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