Induction of Colonic Adenocarcinoma in the Rat by X-Irradiation

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ABSTRACT

In work to determine whether X-radiation could be used to induce tumors of the colon in outbred Holtzman rats, a technique was devised so that only the descending colon could be irradiated with a collimated X-ray beam and tumorigenic exposures in the kilo-Roentgen range were delivered. Ninety male Holtzman rats were divided into 6 exposure groups of 15 animals each. In the first group only the colon was exposed to 2500 R; in the second group the colon was exposed to 3500 R and the exposures were increased by 1000-R increments up through 6500 R; the sixth group was sham irradiated. Animals in each of the irradiated groups developed mucoid adenocarcinomas with the highest incidence (47%) obtained in the 4500-R group.

Histopathologically, the induced tumors bear a close resemblance to human colon adenocarcinoma, and the development of pulmonary metastases in one of the animals with multiple primary tumors demonstrated their malignancy. The usefulness of the animal model is discussed.

INTRODUCTION

Several methods for inducing colonic tumors in rats are known; the 2 most commonly used agents are chemical carcinogens and radiation. The use of chemical carcinogens to induce tumors of the colorectum in various animals has been reported (1, 6, 14). However, the use of these chemical agents is often associated with the simultaneous induction of various types of tumors outside the colon as well as the production of undesirable systemic effects.

Radiation has also been shown to induce tumors of the colon. Lisco et al. (8) in 1947 reported that 75% (6 of 8) rats fed quantities of the β-emitter 91Yt developed carcinoma of the colon 1 to 1.5 years after ingestion. Most of the other reports of radiation-induced colonic cancers involve external whole-body irradiation in which the incidence of large bowel tumors was quite low and in which tumors were induced in many other tissues (3).

The induction of neoplasms in the small intestine of Holtzman rats by selective X-irradiation of only the temporarily exteriorized small bowel was first reported by Osborne et al. (11). Subsequently, Coop et al. (5) described the usefulness of this tumor induction system as an animal model for the study of small bowel cancer. The induced neoplasms were reported to be adenocarcinomas, the tumor type most often observed in the small and large bowel of humans. Histopathologically, the adenocarcinomas in humans and Holtzman rats are very similar. Although the X-ray-induced small bowel adenocarcinoma has been useful as a model for the study of the histopathologically similar human small bowel cancer, the occurrence of this cancer in the small bowel of humans is relatively rare. Except for cancer of the skin, the colon is the most common site of carcinoma in Americans and causes more deaths than any other type of cancer (4). Therefore, an animal model of large bowel cancer would be likely to provide some advantage over the small bowel model. Because localized irradiation of the small bowel will induce tumors in that organ, we believed that this type of approach would also work for the colon. Selective irradiation of only the colon in male Holtzman rats (200 to 250 g) was undertaken to demonstrate this. This procedure removed the possibility of the induction of primary neoplasms in organs other than the colon, and it reduced systemic effects to a minimum. This study was designed to investigate how the level of X-ray exposure affects the incidence of colonic neoplasms and to characterize histopathologically all such induced tumors.

MATERIALS AND METHODS

A procedure was developed that enabled us to irradiate only the colon so that exposures up to several thousand R could be administered with optimal levels of animal survival. This technique was designed specifically to deal with the restraints imposed by the anatomic relationship of the rat colon to the remainder of the abdominal viscera. The descending portion of the colon, the only segment of the colon irradiated in this study, lies along the posterior midabdominal wall and is tightly secured to the posterior abdominal wall by mesentery. It was therefore impractical to exteriorize the colon as has been done in small bowel irradiation (11). Instead, the colon was irradiated in situ.

Each animal was prepared for irradiation by anesthesia with sodium pentobarbital (40 mg/kg). After a midabdominal laparotomy, the small intestine was exteriorized and positioned on a 0.9% NaCl solution-moistened gauze overlying the chest of the rat. The descending colon was then pulled as far from the posterior abdominal wall as its mesenteric attachments would permit. Following this the entire circumference of the descending colon was clamped superiorly and inferiorly with 1.5-inch Dieffenbach serrefine vessel clamps in order to cause hypoxia in the intervening area to be irradiated. At least 2 min were allowed to pass before irradiation began so that sufficient hypoxia would occur and partially protect the subsequently irradiated colon (11).

The animal was then placed on a wooden platform midway between 2 rectangular wooden blocks that ran parallel...
to the long axis of the rat’s body. The blocks acted as supports for the external shielding and also maintained the animal in a static position. Small sheets of lead (2 mm thick) covered by paraffin were then arranged lateral to the colon between the X-ray source and the abdominal viscera. This effectively isolated the colon so that it could be irradiated alone. A second layer of 3.2-mm-thick lead shielding (17 x 24 cm) with a single rectangular aperture was then placed atop the wooden supports, and the aperture was positioned over the colon to be irradiated. This arrangement reduced the exposure to the remainder of the animal to less than 1% of that received by the colon.

As soon as all the shielding was in place, the rig was centered under the X-ray beam (G. E. Maxitron 250, operating at 250 kVp, 30 ma, with 0.25-mm copper and 1-mm aluminum added filtration). The distance from tube target to colon was approximately 25 cm, and the exposure rate to the colon was about 1300 R/min.

This study utilized 90 male Holtzman rats (200 to 225 g) comprising 6 exposure groups of 15 animals each. The colon only of the first group was exposed to 2500 R; in the second group the colon was exposed to 3500 R; in each succeeding group radiation was increased by 1000-R increments up to and including 6500 R. The sixth group was sham irradiated. After irradiation the animals were observed for 7.5 months. Those that died prior to this time were autopsied and checked grossly for signs of tumor growth. At the end of the observation period, all survivors were examined by laparotomy for colon tumors. Those animals without grossly visible evidence of tumors were sacrificed, and representative samples of the irradiated colon were removed for histological examination. Those animals with grossly visible tumors were saved for later transplantation experiments.

RESULTS

Chart 1 relates the percentage of irradiated animals that survived as a function of time following exposure for each of the 5 exposure groups. The desired long-term survival was obtained in the groups that received 2500, 3500, and 4500 R, in which 80, 80, and 93%, respectively, survived the 7.5-month period. The 2 highest exposure groups, 5500 and 6500 R, showed a relatively constant rate of mortality with time with 40 and 33%, respectively, surviving until laparotomy.

The principal cause of death in those animals that died prior to sampling was colonic constriction. Because of the magnitude of the exposures delivered to the colon, much of the regeneration of the irradiated tissue was through fibrous replacement; this was especially true for the higher exposure levels (Fig. 4). This scarring was usually most prominent at the distal margin of the irradiated portion of the colon. Contraction of the scar caused the lumen to narrow significantly and become partially or completely occluded by impacted bowel contents. The affected animals continued to feed, causing the volume of material in the gut proximal to the stricture to increase. The increased volume resulted in an expansion of the irradiated portion of the colon proximal to the stricture. This buildup of material proximal to the constriction led to increasing animal weight while at the same time the animals began to show advanced symptoms of malnutrition. Just prior to death the belly was usually greatly distended due to the enormous size of the colon and the presence of gas in much of the distal small bowel. This entire sequela we have referred to as EBS.3

Chart 2 shows the incidence of EBS in the irradiated animals, up to the time of sampling, as a function of exposure. The frequency varies with exposure. At 2500 R no animals were affected by this condition, while at 3500 R

* The abbreviation used is: EBS, enlarged bowel syndrome.
33% of the animals were affected during the 7.5 months they were observed. A value of 47% was reached with 4500 R; it increased slightly to a peak of 53% with 6500 R. The overall mortality resulting from EBS also varied with exposure. Less than one-half of the animals affected in the 3500- and 4500-R groups died within 7.5 months, while above 4500 R all animals developing EBS within the observation period died.

The number of animals that developed tumors in the colon during the observation period was dose dependent. In Chart 3, the incidence of colonic neoplasms (in percentage of animals at risk) is plotted as a function of X-ray exposure. The incidence increased as a function of exposure and reached a maximum of 47% in the 4500-R-exposure group. Thereafter, increasing exposure caused such a significant drop in animal survival that the tumor incidence was only 13% in both the 5500- and 6500-R groups (in which 2 of 6 and 2 of 5 surviving animals, respectively, developed tumors).

Histopathologically, the induced tumors were well-differentiated mucoid adenocarcinomas, the only type of primary colon tumor found thus far in the irradiated animals. In general the following description applies to all tumors found thus far. The tumor cells ranged from well-differentiated cells forming irregularly shaped acini to poorly differentiated cells that were unable to form any gland-like structures. The tumor cells making up the acini were found both in submucosal tissue and within the muscle wall. They varied from normal-appearing columnar to low cuboidal cells. The overall morphology of the low cuboidal cells was significantly abnormal with markedly increased nuclear-cytoplasmic ratios as compared to normal colonic mucosa. The poorly differentiated tumor cells, unlike their better differentiated counterparts, had lost the ability to form glands. Most grew singly or in small groups separated by desmoplastic connective tissue. The whole area of tumor growth was attended by a chronic inflammatory response with numerous lymphocytes and plasma cells (Figs. 1 to 3).

**DISCUSSION**

The study of colon carcinoma in humans has been facilitated by the development of animal models. The majority of these experimental tumors are induced by chemical agents, the colorectal specificity of which has been questioned by many investigators (6, 10, 13, 15, 16). Even the compound symmetrical 1,2-dimethylhydrazine, one of the agents thought to be most specific for inducing neoplasms of the colorectum, has been shown to induce tumors in many other organs including the small intestine, kidney, liver, and ear canal (10, 13, 15). The presence of multiple tumor types in single animals would be expected to alter the immune response of the animal in comparison to those animals with 1 tumor type. In addition most of these chemical agents produce untoward systemic effects when the route of administration allows the agent access to the general circulation (i.v., i.p., and s.c.). For example, single toxic doses of 1,2-dimethylhydrazine administered s.c. induce mild centrilobular necrosis of the liver in rats and mice, as well as inducing monomer formation in rat liver polysomes. Additionally, the incorporation of [%H]leucine into rat liver protein is inhibited (7).

Localized irradiation of only the descending colon of rats, as a means of colon tumor induction, eliminates the possibility of developing tumors in organs other than the colon as well as reducing to a minimum any systemic effect of the induction procedure. Because only the descending colon is treated, both normal and neoplastic colon tissue can be obtained from the same animal. Such a situation can greatly improve the quality of immunohistochemical reagents prepared against these tumors. For example, antitumor antisera prepared in rabbits can be absorbed with the corresponding normal-tissue preparation from the same animal. This eliminates the possibility of alloantigenic differences existing between the rat bearing the tumor and the normal tissue donor rat. This can be especially important with an outbred rat stock such as the Holtzman rat.

The development of pulmonary metastases in 1 of the rats exposed to 3500 R indicated that these induced colonic adenocarcinomas possess some degree of malignancy. This further correlation with the spread of colon carcinoma in humans helps emphasize the usefulness of these radia-
tion-induced neoplasms as a model of human colorectal cancer (Figs. 5 and 6).

On comparison of the survival data (Chart 1) with the tumor incidence data (Chart 3), it is apparent that X-ray exposures in the range of 4500 R will give the highest tumor induction rate (47%) with optimal animal survival (93%). Below 4500 R the survival rate is still good (80%) but tumor incidence is lower (20 to 40%). From 5500 R upward the animal survival rate is so low (33 to 40%) that even if all the surviving animals were to develop tumors the overall incidence would still be less than those exposed to 4500 R. The data therefore suggest that colon irradiations, for the purpose of developing a pool of tumor-bearing animals for study of the resultant tumor tissue, would be best carried out at the 4500-R level. With this regimen at least one-half of the animals surviving 7.5 months should develop colonic adenocarcinomas.

Previous studies of radiocarcinogenesis in organs other than the colon have shown several correlations with our own model. In 1969 Maldague reported a study of radiocarcinogenesis in the rat kidney in which tumors were induced by an \textit{in situ} irradiation technique with a specially collimated X-ray beam of quality similar to that of the beam used in our study (9). One year earlier Burns \textit{et al.} (2) had reported on the induction of skin tumors in rats and mice by either \textalpha{}-particle or electron beam irradiations. Both of these previous investigations, as well as our own study, showed that the tumor incidence was directly related to the dose delivered to the target organ. At lower doses few or no tumors were induced. As the doses were increased above those causing minimal tumor incidence, the rate of tumor induction increased. In each case as the dose is increased further a peak induction rate is reached; thereafter increasing doses result in a reduction in tumor incidence. In the present study the optimal colon tumor induction rate is reached at an exposure of 4500 R at which level 47% of animals developed cancers. Maldague's data showed that malignant tumors of the kidney are not induced by doses of 570 rads or less. As the dose is increased above 855 rads, the incidence of cancer rises, reaching a peak of 63% with a dose of 1710 rads. Above this dose the tumor incidence begins to decline, being only 16% at a dose of 7125 rads. Likewise Burns \textit{et al.} reported that the incidence of skin tumors was also dose dependent, reaching a peak at 1050 rads of 37-meV \textalpha{}-particles, or 2880 rads of 0.37-meV (effective energy) electrons. Histopathologically, it was found that carcinomas were the most frequent cancers induced in these studies. (The only exception was that, in the kidney, doses in excess of 7000 rads produce mainly sarcomas; however, less than 16% of animals irradiated at these doses develop cancers.) In the present study all carcinomas of the colon were well differentiated. The data on skin tumors, when all doses are combined, show that 67% of the tumors are differentiated and 26% are undifferentiated or anaplastic. Maldague's results relative to kidney irradiations showed that below 1140 rads the differentiated type predominated while at doses between 1710 and 2850 rads undifferentiated carcinomas were the most prevalent. These histopathological findings tend to indicate that, for doses in the range of optimal tumor production under the conditions imposed in each of these studies, the epithelial cells of the skin, kidney, and colon are more sensitive to malignant change than are the other cell types present within the irradiated tissues. It remains to be resolved whether the epithelial cells of these organs, relative to the other cell types present, are more susceptible to malignant transformation by a given dose of radiation or whether transformations are simply more easily expressed due to the more rapid proliferative rates found among these epithelial cells.

As a final note, in the course of histological studies of the irradiated colons from those animals not displaying grossly visible tumors upon examination, many areas of pathological bone formation were found. These foci of heterotopic ossification were present in many of the irradiated colons regardless of whether cancer had developed. Heterotopic ossification in primary or metastatic epithelial neoplasms is rare (12), and no reports could be found in the literature of induced bone formation in the colon by radiation. We plan to investigate this effect of high-dose radiation on the rat colon more thoroughly in the future.

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**REFERENCES**

Rat Colon Carcinogenesis

Fig. 1. An intraluminally growing tumor of the descending colon from a rat exposed to 4500 R, 232 days before sacrifice.
Fig. 2. A typical radiation-induced, well-differentiated mucoid adenocarcinoma. × 18.
Fig. 3. Higher-power view of specimen from Fig. 2, showing both the well- and poorly differentiated growth of colon tumor cells. Note many large mucin-filled glands separated by desmoplastic connective tissue. × 37.
Fig. 4. A portion of the descending colon of a rat exposed to 3500 R, 232 days prior to sacrifice. The extensive fibrosis narrowed the intestinal lumen, leading to its partial obstruction. Note large sclerotic vessel (directly above figure number), which is a typical manifestation of late radiation damage. × 30.
Fig. 5. Lung tissue from a rat with multiple radiation-induced colon carcinomas. A large cord of metastatic tumor cells runs through the center of the lung tissue. × 40.
Fig. 6. Higher-power view of specimen from Fig. 5 showing mucin-filled glands formed by the metastatic colon tumor cells. × 64.
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