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Influence of Dose Rate on Carcinogenesis Resulting from X-Ray, ^{113m}In, and ^{198}Au Irradiation

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The potential hazards from internally administered radionuclides used in nuclear medicine are usually compared with one another and with diagnostic x-rays on the basis of the absorbed dose in rads, with no regard to the dose rate of the radiation. This study compared the carcinogenic potential of a dose of 250 rads delivered at different dose rates to rat livers by x-ray, ^{113m}In, and ^{198}Au. The chemical carcinogen N-2-fluorenyldiacetamide was administered after irradiation to reduce the latent period and increase the number of radiogenic liver tumors. No significant difference in tumor incidence was observed among groups of animals treated with either ^{198}Au, ^{113m}In, or x-irradiation.

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The influence of the dose rate on the amount of biologic damage caused by absorbed radiation doses encountered in nuclear medicine has received minimal attention (*1,2*) despite radiobiologic experience that given radiation doses generally produce less damage when delivered at lower rates. When the potential hazards from internally administered radionuclides are compared with each other and with diagnostic x-rays, the comparisons are usually made on the basis of absorbed dose with no regard for differences in dose rate. However, the irradiation time for any given dose may vary from fractions of a second with diagnostic x-rays to hours, days, or weeks with radionuclides.

In a previous report (*3*), we used rat liver as a model to compare the effectiveness of a dose of x-rays (given in minutes) with the same dose given at lower rates from the radiocolloids ^{113m}In (1.7 hr) and ^{198}Au (2.8 days) for the production of chromosomal aberrations. In the range 125–1000 rads, irradiation with x-rays or ^{113m}In was approximately twice as effective as ^{198}Au in inducing chromosomal aberrations (bridges). The relationship of such radiation-induced aberrations to induction of cancer is not clear, but there is some evidence that such a relationship may exist (*4*). This study compares the carcinogenic potential of a dose of 250 rads delivered at different dose rates to the rat liver by x-ray, ^{113m}In, or ^{198}Au.

MATERIALS AND METHODS

Experimental animals. A total of 216 male Charles River CD rats, weighing approximately 200 gm each, was used in these studies. The animals were maintained on a constant light–dark cycle with food and water always available.

X-irradiation. The animals received x-ray doses of 250 rads (280 kVp, 1.55 mm Cu HVL) to their livers at an exposure rate of 160 R/min, verified with calibrated ion chambers and lithium fluoride measurements in the carcasses of dead animals and phantoms. Lead strips, which reduced the radiation level to 2.5% of its unshielded intensity, covered the animals' bodies except for the hepatic region. The ani-

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mals were irradiated in individual containers between 9:30 and 10:00 a.m.

Radiopharmaceuticals. The animals received intravenous injections of either colloidal ^{198}Au or colloidal ^{113m}In. These radionuclides were chosen because their half-lives and, consequently, their decay rates are quite different, while their radiations are similar (*5*). Thus, quite similar radiations can be delivered at dose rates that vary considerably, making an analysis of the role of the dose rate possible. The major gamma-photon energies for ^{198}Au and ^{113m}In are, respectively, 0.412 and 0.393 MeV. Although ^{113m}In does not undergo beta emission, approximately one-third of its gamma photons are internally converted to electrons whose energies (~0.37 MeV) are similar to the average beta energy of ^{198}Au (0.317 MeV). Because there is no biologic elimination of these radiocolloids by the liver, the effective half-times are equal to the physical half-lives. Details of colloid preparation, distribution of radiocolloids, injection technique, calculation of absorbed dose, and relative dose rates have been reported previously (*3*). We chose a hepatic radiation dose of 250 rads with both the radiocolloids and x-rays because of reports that tumor incidence curves tend to reach a maximum at around 250 rads and may even decrease as the dose is raised to higher levels (*6*).

Cocarcinogen. In an effort to stimulate the production of radiation-generated hepatic tumors and to reduce the latent period, the animals were given oral doses of the chemical carcinogen N-2-fluorenyldiacetamide (2-FAA) after the irradiation. This agent has been shown to be a potent cocarcinogen with radiation (*7*). Beginning 1 month after x-irradiation or radiocolloid injection, the 2-FAA was administered through a stomach tube (16.5 mg of 2-FAA in 0.5 ml of cottonseed oil) three times each week for 3 weeks, followed by 1 week without 2-FAA feeding. This cycle was repeated four more times, resulting in a total intake of 750 mg of 2-FAA per rat. Thus, the 2-FAA was administered over 5–23 weeks from the time of x-irradiation or injection of the radiocolloids. Stomach feeding (as opposed to adding 2-FAA to the diet) was used to ensure that each rat in every group received the same quantity of the chemical, thus facilitating comparison of the effects of the radiation regimens. A summary of the experimental design is given in Table 1.

Autopsy and tissue processing. Longevity data were kept on all experimental animals. The animals were usually followed until death, but occasionally, when rats were moribund, they were killed with ether. All animals still alive at 52 weeks were killed. Autopsies were performed on all but two of the 216 animals, and tissue suitable for histopathologic diagnosis was obtained from all but four of them. The tissues for histopathologic study were fixed with 10% buffered formalin and embedded in paraffin, and sections were stained with hematoxylin–eosin. About 30 min before they were killed, most of the animals were injected intravenously with 3 mg of rose bengal dye to facilitate gross examination of the livers for nodules. By this method (*8*), lesions usually were either untinted or a lighter pink than the adjacent bright-pink normal liver. Using the same criteria as Reuber (*8*), all gross lesions larger than 3 mm were studied histologically. In some animals, India ink was injected intravenously to aid in differentiating histologically between hyperplastic nodules and hepatomas, since functioning Kupffer cells are not detectable in hepatomas with this method (*8*). We have been guided in histopathologic identification of the tumors by the work of Reuber (*8*), Stewart and Snell (*9*), and Firminger (*10*).

RESULTS

Because of the toxic effects of the 2-FAA (Groups 2–5, Table 1), approximately 25% of the rats were dead by the end of this treatment. A comparison of animal survival curves using a method of life table analysis (*11*) showed no statistical difference ($p > 0.05$) in survival among all these groups. Only one animal receiving only the carrier oil (Group 1) died during the 52-week interval.

The results of the gross and histopathologic examination of the animals' livers are given in Table

TABLE 1. SUMMARY OF THE EXPERIMENTAL DESIGN

Group	No. of rats	Treatment
1	28	Oil*
2	40	2-FAA†
3	40	^{198}Au→2-FAA‡
4	38	^{113m}In→2-FAA‡
5	70	x-ray→2-FAA‖

* The animals received 0.5 ml of oil carrier three times each week through a stomach tube for 3 weeks, followed by 1 week with no oil feeding. This cycle was repeated five times.

† The animals received 16.5 mg of 2-FAA in 0.5 ml of oil carrier three times each week by stomach tube for 3 weeks, followed by 1 week with no treatment. This cycle was repeated four more times. Thus, each animal received a total of 750 mg of 2-FAA over a 19-week period.

‡ Sufficient radiocolloid was injected to deliver 250 rads to the liver from complete decay (99% decay occurs in 11 hr for ^{113m}In and in 18 days for ^{198}Au). The 2-FAA treatments (see above) were begun 4 weeks after radiocolloid injection.

‖ X-irradiation at 160 R/min delivered 250 rads to the liver. The 2-FAA treatments (see above) were begun 4 weeks after x-irradiation.

TABLE 2. INCIDENCE OF HEPATIC TUMORS

	Group	Tumor incidence*				
		Up to 30 weeks	30–39 weeks	40–51 weeks	At 52 weeks	Total
1.	Oil	0/1	0/0	0/0	0/27	0/28
2.	2-FAA	0/14	0/5	0/6	4/15	4/40
3.	^{198}Au	0/9	0/6	4/10	6/15	10/40
4.	^{113m}In	0/8	3/8	1/3	11/19	15/38
5.	X-ray	0/18	5/20	5/12	10/20	20/70

Results were analyzed by the life table method and the Breslow test for failures. All groups *not* underscored by the same line are significantly different ($p < 0.05$):

4 5 3 2 1

* Incidence is expressed here as the number of rats with tumors over the number of rats tested.

2. Life table analysis and the Breslow test (*11*) for failures showed no significant difference in tumor incidence among those receiving either x-irradiation or the radiocolloids (Groups 3–5). Comparison of the tumor incidences between Groups 3 and 4 (^{198}Au and ^{113m}In treatment) was suggestive of a difference, but only at the $p = 0.1$ level. Some animals had more than one liver tumor (usually in different liver lobes), but analysis based upon the total number of tumors induced showed no significant differences among Groups 3, 4, and 5. The liver tumors found were mainly poorly to well differentiated hepatomas, with a small number (14%) of cholangiocarcinomas.

DISCUSSION

The fact that low LET radiations given over a period of weeks or months are less carcinogenic than similar radiations given in a matter of minutes or hours is well documented (*12–16*). This general relationship of dose rate to carcinogenesis holds well, and the reported exceptions in which lower dose rates are more effective than higher ones pertain to particular tumor types in particular experimental animals (*13,16*). The mechanisms underlying the reduced carcinogenic efficiency of lower dose rates could include: more efficient intracellular recovery, less immunosuppression, and age-dependent loss of sensitivity over prolonged irradiation intervals (*12,17*). Since the greatest protraction of radiation exposure in our experiments is only about 3 weeks (99% of the absorbed dose from ^{198}Au occurs in 18 days), age-dependent loss of sensitivity is probably minimal. Dose rate differences related to changes in immunosuppression should also be minimal because the animal's body is shielded during hepatic x-irradiation and because most of the radiocolloid is localized in the liver, resulting in intense local irradiation (*4*).

Chemical carcinogens as cocarcinogens (or "promotors") have been reported to enhance radiation tumorigenesis in the liver (*7,18,19*), and our results are consistent with these observations. In using a chemical cocarcinogen (2-FAA) to stimulate the production of hepatic tumors and to decrease the latent period, we chose to administer the same amount of the chemical to all the irradiated animals. Although the chemical alone produced some tumors (Table 2), the significant differences in tumor incidence above this level in the irradiated animals should indicate the effects of the irradiation.

Although we have previously shown a dose-rate-dependent difference in the ability of radionuclides to produce chromosomal aberrations (*3*), we were unable to show a similar significant difference in carcinogenic potential in the dose rate range between ^{198}Au, ^{113m}In, or x-irradiation (Table 2) under the conditions of this experiment. These results are also useful in assessing the potential carcinogenic hazard associated with the more widely used ^{99m}Tc, since its half-life (6 hr) lies between those of ^{113m}In and ^{198}Au.

We recognize that our information has limitations, that the radiation doses encountered in nuclear medicine are usually less than those used in our experiments (by a factor of 10–100), and that extrapolation from animals to man is always risky. Nevertheless, we believe this experiment provides evidence indicating that the higher dose rates associated with the use of newer short-lived radionuclides in nuclear medicine do not represent an increased carcinogenic hazard to the patient. Our results are in no way to be regarded as the final word, but they are an encouraging indication.

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