CORRELATION OF DOSE RATE AND BIOLOGICAL EFFECT IN RAT LIVER CELLS 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 each other and with diagnostic x-rays on the basis of the absorbed dose in rads with no regard to the dose rate of the radiation. Using the production of chromosomal aberrations (bridges) in rat liver cells as an endpoint for biological damage, we compared the effectiveness of irradiation when the same total dose was delivered rapidly with x-ray or at lower dose rates with radioactive colloids of ^{113m}In or ^{198}Au. Because in the adult liver there is little cell division with associated loss of damaged cells, chromosomal aberrations are retained when irradiation is either rapid or protracted. However, when an urgent demand for cells occurs, such as after surgical removal of a major portion of the liver, the liver responds by undergoing rapid proliferation which reveals this chromosomal damage. In a range of dose between 125 and 1,000 rads, irradiation with x-rays or ^{113m}In was approximately twice as effective as ^{198}Au in inducing chromosomal bridges. The relationship of damage (bridges) to dose appears to be linear in each case and extrapolation to low doses suggests a similar time-dose effect, but direct proof of this is lacking.

The significance of dose rate on the amount of biological damage caused by absorbed doses and dose rates encountered in nuclear medicine has received minimal attention (*1–3*). Radiobiologic experience has shown that for a wide variety of biologic effects, the extent of damage for a given dose of low LET radiation decreases as the dose rate increases. The potential hazards from internally administered radionuclides used in nuclear medicine are compared with each other and with diagnostic x-rays on the basis of absorbed dose in rads with no regard for differences in dose rate. Yet the irradiation time may vary from seconds with diagnostic x-rays to days or weeks with radionuclides.

This study compared the biological damage with an in vivo model system when the same total dose was delivered rapidly with x-irradiation or at lower dose rates with radionuclides (2.7-day ^{198}Au and 1.7-hr ^{113m}In).

MATERIALS AND METHODS

Model system. Liver cells of the rat were used as the model system for these studies. Cell division in the normal adult liver is infrequent; however, after partial hepatectomy the system responds by increased proliferation. Radiation doses of the order of 500 rads produce no obvious histologic effect in the adult liver. If part of the liver is removed after irradiation, large numbers of the remaining cells will divide within 25–36 hr, and radiation damage is then revealed as broken chromosomes, abnormal mitotic patterns, and chromosomal bridges. This provides an excellent system for dose-rate studies since damage produced when irradiation is either rapid or protracted is retained in the intact adult liver and expressed at a later time when partial hepatectomy is followed by cell proliferation.

Experimental animals. Approximately 450 male Charles River CD rats, 6–8 weeks old and weighing between 125 and 200 gm, were used in these experiments. Only animals weighing greater than 180 gm were used for ^{198}Au treatment because of possible loss of chromosome aberrations during the irradia-

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tion period (*4*). The animals were maintained on a light-dark cycle with food and water always available.

X-irradiation. Animals received doses of x-ray from 125 to 500 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 levels to 2.5% of its unshielded intensity shielded their bodies except for their hepatic regions. The animals were irradiated in individual containers between 9:30 and 10:00 am.

Radiopharmaceuticals. Animals received intravenous injections of either ^{198}Au or ^{113m}In as the colloid. These radionuclides were chosen because their half-lives are quite different, yet their radiations are similar (*5*). The major gamma-ray 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 rays are internally converted to electrons whose energies (~0.37 MeV) are similar to the average beta energy of ^{198}Au (0.317 MeV). Radiocolloid gold was obtained from a commercial supplier (E. R. Squibb and Son) while the indium radiocolloid was made using a modification of the technique of Bruno (*6*). The eluate from the ^{113}Sn-^{113m}In generator (New England Nuclear) was concentrated by evaporation, and reagent volumes were kept to a minimum (the gelatin concentration was always less than 0.5%). The ^{113m}In-colloid was autoclaved as the final step in the preparation.

Distribution of radiocolloids. Blood levels and organ distribution studies were performed in 90 animals to provide the biological data necessary for internal dose calculations (Table 1). Homogeneity of uptake within the liver was confirmed by assaying small portions and by autoradiography. No evidence of biologic elimination of either radiocolloid was seen.

Radiocolloid treatment. Radiocolloids were injected into the surgically exposed femoral vein to assure complete intravenous delivery of the administered activity. Syringes containing the radiocolloids were assayed immediately before and after injection to assure delivery of the calculated activity. Before treatment, aliquots of the radiopharmaceutical from each batch were injected into two or three animals to assure for each experiment that the distribution of activity in the organs did not deviate by more than 5% from the values used in the dose calculations. All injections were made between 9:30 am and 12:00 noon. Some animals were pretreated with radiocolloids which had been allowed to undergo decay to negligible levels of activity. This treatment with "cold" colloids was used to determine whether there were chemical effects related to the colloidal materials.

TABLE 1. DISTRIBUTION OF RADIOCOLLOIDS

Radiocolloid	Percentage of administered activity with standard error		
	Liver	Spleen	Carcass
^{113m}In	84.4 ± 1.2	1.1 ± 0.3	13.9 ± 0.6
^{198}Au	93.0 ± 1.1	0.9 ± 0.2	5.4 ± 0.4

Internal absorbed dose calculation. The absorbed dose to the liver of the rats used in these studies was calculated using the methods recommended by the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine (*7*) and the biological distribution data obtained in these studies (Table 1). The 90 animals used in the organ distribution studies were weighed and their livers removed and weighed at sacrifice. The liver constituted an average of 5.14 ± 0.44% (mean ± s.d.) of the body weight. Since the biological disappearance half-time of the radiopharmaceutical in the blood was approximately 0.1 hr, instantaneous uptake by the various organs can be assumed. It was also assumed that the effective half-life for both radionuclides was equal to their physical half-life since there was no biological elimination of the radionuclide from the liver. The cumulative activity in the liver per curie injected was 87 μCi/hr for ^{198}Au and 2.0 μCi/hr for ^{113m}In. For the total body, the cumulative activity per microcurie injected was 93 μCi/hr for ^{198}Au and 2.4 μCi/hr for ^{113m}In.

The dose to the liver results from penetrating and nonpenetrating radiations from activity in the liver, and penetrating radiation from activity in the remainder of the body which was assumed to be uniformly distributed. The decay scheme data used for the dose calculations were derived from MIRD Supplement No. 2 (*5*). It was assumed that the liver and the total body of the rat can be represented by ellipsoids whose axes are in the ratio of 1:2:4. Using this assumption, the absorbed fractions for a uniform distribution of activity in ellipsoids of appropriate sizes were obtained from MIRD Supplement No. 5 (*8*). For an injected dose of 1 μCi/gm of body weight, the dose to the liver for complete decay of ^{113m}In was 12 rads and for ^{198}Au it was 1,200 rads.

Surgical procedure. Removal of approximately 70% of the liver (*9*) was performed under light ether anesthesia. Operations were always performed between 10:00 am and 12:00 noon.

TABLE 2. SUMMARY OF THE EXPERIMENTAL DESIGN

Group	Material injected	Type of irradiation	Duration of irradiation* (day)
1	None	None	0
2	Stable gold colloid	None	0
3	Stable indium colloid	None	0
4	None	x-ray	†
5	Stable gold colloid	x-ray	†
6	Radioactive gold colloid	^{198}Au	2
7	Radioactive gold colloid	^{198}Au	5
8	Radioactive gold colloid	^{198}Au	12
9	Radioactive gold colloid	^{198}Au	18
10	Radioactive indium colloid	^{113m}In	1

* Rats hepatectomized after irradiation completed for radionuclides and 24 hr after x-ray irradiation. Rats injected with "cold" radiocolloid, i.e., radionuclide allowed to decay to negligible level, were hepatectomized 24 hr after injection. All rats sacrificed 28 hr after hepatectomy .

† Exposure rate was 160 R/min.

Histologic analysis. Animals were killed with ether 28 hr after partial hepatectomy. After sacrifice the livers were rapidly excised and placed in 10% buffered formalin for 24 hr. Histologic sections obtained from wax-embedded tissues were stained with Feulgen and fast green. Although various kinds of chromosomal abnormalities were seen, only chromosome bridges in anaphase and telophase were recorded because objective criteria for scoring these are more readily established. Further, although bridges represent only a fraction of the damage, it is probably a constant portion (*10*). The results are expressed as the percentage of anaphases and telophases having bridges. Where possible, a total of 100 anaphase and telophase figures were recorded from each slide. The peripheral portions of the sections were not counted because of the rapid drop in dose at the surface of a volume source containing a beta-emitting radionuclide (*11*).

Experimental design. The experimental design included ten groups of animals as shown in Table 2.

RESULTS

The results from experimental groups 1, 4, 9, and 10 are summarized in Table 3. We were unable to obtain data at doses greater than 400 rads with ^{113m}In because of the large quantities of activity necessary to deliver the higher doses. Control levels (Group 1) of bridges consistently ranged between 0.2 and 0.3% in these young animals. The results indicate that irradiation with x-rays and ^{113m}In is approximately twice as effective ($p < 0.01$) as ^{198}Au in inducing chromosomal bridges in the dose range studied, whereas no statistical difference could be demonstrated between results obtained after irradiation with ^{113m}In or x-rays. The dose-response data were fitted to three models, $Y = a + bD$, $Y = a + cD^2$, and $Y = a + bD + cD^2$ with the linear model providing the best fit in all cases where Y = response and D = dose. The relationship of damage to dose appears to be linear (correlation coefficients for x-ray, ^{113m}In, and ^{198}Au were respectively 0.95, 0.99, and 0.96) with no statistical evidence to indicate that the lines do not pass through the origin. Computer-fitted regression curves were determined from the data and are shown in Fig. 1.

TABLE 3. PERCENTAGE OF ANAPHASE AND TELOPHASE BRIDGES* IN HEPATOCYTES AFTER IRRADIATION

Dose (rads)	X-ray	^{113m}In	^{198}Au
125	4.6 ± 0.9	4.7 ± 1.6	2.6 ± 0.9
250	10.6 ± 1.6	9.9 ± 2.9	5.8 ± 1.1
	11.6 ± 2.3		4.7 ± 1.6
400		14.2 ± 3.6	
500	22.3 ± 1.7		9.5 ± 1.0
	16.2 ± 1.7		7.4 ± 0.6
	21.3 ± 1.4		13.0 ± 1.7
			9.3 ± 1.6
1,000			20.2 ± 1.9
			17.3 ± 1.2

* Listed as the mean and standard deviation. The number in each group varied between 15 and 25 animals. Control levels of 0.25–0.3

Because of the possible loss of bridges (such as occurs from cell division) during the 18-day period between injection of the ^{198}Au-colloid and partial hepatectomy, the level of aberrations were measured in 20 animals at intervals before and at the completion of irradiation. Animals were injected with sufficient ^{198}Au to deliver 250 rads to the liver from complete decay but were partially hepatectomized (irradiation continuing in the liver stump after sur-

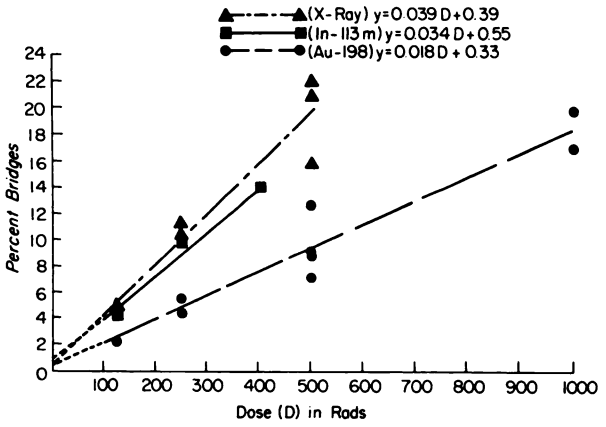

FIG. 1. Production of chromosomal bridges at various dose levels after irradiation with x-ray, ^{113m}In, and ^{198}Au.

gery) and sacrificed at specific intervals up to this time (Groups 6, 7, and 8). Compared with the yield of 4.1% at 18 days (Group 9), animals killed at 2, 5, and 12 days after injection produced aberration levels (1.9, 2.8, and 3.8%, respectively) in proportion to the fraction of the total dose delivered (substantiating that significant loss did not occur). Finally, animals pretreated with the "cold" colloids (Groups 2 and 3) had an incidence of chromosome bridges not different from controls (Group 1). The incidence of aberrations when pretreatment with "cold" gold colloid was followed by x-irradiation (Group 5) was not different for x-irradiation alone (Group 4).

DISCUSSION

The frequency of anaphase abnormalities (usually presented as bridges, bridges plus fragments, etc.) has been widely used as a measure of radiation damage in many biologic systems (*10,12–15*). Although aberrations such as bridges represent only a fraction of the damage induced, it is probably a constant portion. Conger and Curtis (*10*) have reported that anaphase bridges initially increased with dose more or less linearly to a maximum and then actually began to decline due to bridge loss processes at higher doses. Within the dose ranges studied, we saw no evidence of bridge loss since a linear relationship between dose and damage provided a "best-fit" for our data.

In previous experiments (*16*), we demonstrated no difference in the number of chromosomal bridges in liver cells when the body weights of the animals at time of x-irradiation varied between 70 and 260 gm. Although a reduction in the number of aberrations over a period of weeks is observed in smaller animals, aberrations persisted undiminished for up to 7 weeks in animals weighing approximately 200 gm at irradiation. Thus the adult rat liver proved to be well suited to our dose-rate studies since it is necessary to be able to compare *total* damage even when the treatment interval is protracted (as in the case of ^{198}Au). Further, damage which is relatively "permanent", such as chromosomal aberrations, represents the most important change because of the speculation that chromosome damage may precede the development of neoplasia, and also may predispose to malignant changes due to increased susceptibility to infection by an oncogenic virus (*17*).

Important relationships between dose rate and damage have been demonstrated in a variety of systems. Using external whole-body irradiation, Nowell, et al (*18*) have demonstrated that in mouse liver 500 rads of high dose-rate x-irradiation (1,800 rads/hr) produced more than twice as many cells with chromosomal aberrations when compared with 1,000 rads of low dose-rate gamma rays (1.45 rads/hr). Liniecki, et al (*19*), using cultures of human lymphocytes irradiated in vitro with gamma-ray doses varying from 134 to 355 rads, observed a decrease in the number of dicentric aberrations when the dose rate fell below 12–24 rad/hr. Brewen and Luippold (*20*), using human lymphocytes irradiated in culture by acute x-ray or chronic gamma irradiation (6,000 vs. 4.4 rads/hr), also demonstrated a significant dose-rate effect for doses as low as about 100 rads. Pfannenstiel (*21*) had concluded from clinical observations on patients that fractionated x-ray is about twice as effective as protracted continuous ^{131}I irradiation in the treatment of thyrotoxicosis. Our demonstration that irradiation with rapid x-ray or short-lived ^{113m}In is twice as effective as ^{198}Au in inducing chromosomal aberrations in vivo (in the dose range studied) is in agreement with these observations. The dose-rate level for the x-irradiation and initial radionuclide levels for specific doses are given in Table 4. In the case of the radionuclides, the dose rate decreases with decay (halved after the first half-life, etc.).

Brooks, McClellan, and Benjamin (*22*) have shown no effect of dose rate on the frequency of chromosome aberrations (mainly dicentrics) in hamster liver after intraperitoneal injection of ^{144}Ce-^{144}Pr. Cells were stimulated to divide by partial hepatectomy. By controlling the duration of irradiation and the injected dose, they were able to study the frequency of aberrations/cell/rad over a wide range of dose. Differences in dose rates over a range of 0.08–10.4 rad/hr had no effect on the efficiency of production of aberrations in this study. A comparison of our results with those of Brooks, McClellan, and Benjamin are difficult since their data are a result of constant dose rates while ours were continually varying with decay of the radionuclides. The range of dose rates encountered in their study are similar to our initial dose rates with ^{198}Au. We also saw no dose-rate effect on the efficiency of the production of aberrations for a specific agent. That is, the effi-

TABLE 4. APPROXIMATE INITIAL DOSE RATE IN RADS/HR FOR SPECIFIC DOSES (RESULTING FROM X-IRRADIATION OR FROM COMPLETE DECAY OF RADIONUCLIDES)

Dose (rads)	X-ray	^{113m}In	^{198}Au
125	9,600	58	1.5
250	9,600	116	3.0
400		186	
500	9,600		6.0
1,000	9,600		12.0

ciency did not change when the initial dose rates of ^{198}Au varied between 1.5 and 12 rads/hr or when the dose rates varied between 58 and 186 rads/hr with ^{113m}In.

The doses encountered in diagnostic nuclear medicine procedures are usually less than those used in our experiments by a factor of at least ten. It has been the assumption of groups such as the NCRP (*23*) that the degree of effect is dependent not only on dose rate but also on the dose level for low LET radiations. The NCRP assumes an independence of degree of effect for doses of 6 rads of low LET radiation delivered in one short exposure and 5 rads spread over a year. Others (*24*) have suggested that a single exposure of 25 roentgens or less could be treated as equal in effect to the same exposure to low dose-rate irradiation. Our results do not supply direct information at these low dose levels. This independence of effect on dose rate at low doses (*3,23,24*) with low LET radiation is based upon the following assumption: that there is a quadratic dose-effect relationship at high dose rates whereas there is a linear dose-effect relation at low dose rates. Thus at lower doses the curves of dose versus effect come together. Our experiment using high dose-rate x-ray as well as high and low dose-rate internal emitters resulted in linear dose-effect relationships for all in the dose range studied. Extrapolation of these curves to lower doses (a risky process at best), however, does not rule out the possibility of dose-rate effects at lower doses.

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