Morphological Transformation of C3H 10T1/2 Cells by ^{99m}Tc-Cardiolite

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The induction of in vitro morphological transformation in C3H 10T1/2 cells by ^{99m}Tc-Cardiolite (contents of Cardiolite kit [hexakis(2-methoxyisobutylisonitrile) and other components] plus ^{99m}Tc generator eluate) was examined. **Methods:** Cells were grown for 48 h in the presence of ^{99m}Tc-Cardiolite or decayed ^{99m}Tc-Cardiolite (^{99m}Tc-Cardiolite after 1 wk of storage), and cell survival and transformation were assessed by the colony-forming and focus assays, respectively. X-ray was used as a reference for radiation effects, and 20-methylcholanthrene was used as a positive control for focus formation. **Results:** Exposure of cells to ^{99m}Tc-Cardiolite results in a transformation frequency that is not significantly different from that induced by the volume equivalent of decayed ^{99m}Tc-Cardiolite. The number of foci per viable cell increases linearly from $\sim 0.17 \times 10^{-4}$ in the untreated control to 1.7×10^{-4} at 37 kBq/mL and 30×10^{-4} at 1100 kBq/mL ^{99m}Tc-Cardiolite or its decayed ^{99m}Tc-Cardiolite volume equivalent. Furthermore, exposure of cells to low extracellular concentrations of ^{99m}Tc-Cardiolite or decayed ^{99m}Tc-Cardiolite (cell survival, ≥88%) induces an ~20-fold greater number of transformants per viable cell than that observed after 0.5 Gy x-irradiation, a dose that causes the same level of toxicity. **Conclusion:** Radioactive and decayed ^{99m}Tc-Cardiolite induce morphological transformation of C3H 10T1/2 cells in vitro. The underlying mechanism does not seem to be related to the radiation effects of decaying ^{99m}Tc but to chemical(s) present in the ^{99m}Tc-Cardiolite kit.

Key Words: low-energy electrons; ^{99m}Tc; ^{99m}Tc-Cardiolite; ^{99m}Tc-MIBI; neoplastic transformation

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The response of mammalian cells and tissues after exposure to internally deposited radiopharmaceutical can be attributed to radiation-induced ionizations and excitations, nuclear recoil, chemical transmutations, and local charge transfer. These biologic responses are of interest to the practitioner of nuclear medicine from 2 perspectives. In the first instance are the effects observed when moderately large cytotoxic amounts of radioactivity are administered for therapeutic purposes. In the second instance are the long-term carcinogenic and reproductive effects of radiation that may possibly become manifest after the administration of smaller doses of radioactivity for diagnostic purposes (*1*). Whereas for therapeutic purposes it is necessary to kill or alter the function of many cells, for carcinogenesis it is generally essential to alter only 1 or a few cells to initiate the process. Late effects are manifest only in those cells that survive the initial insult while retaining some memory of the exposure.

Several radionuclides used in diagnostic nuclear medical practice, including ^{67}Ga, ^{99m}Tc, ^{111}In, ^{123}I, and ^{201}Tl, decay with the emission of a cascade of low-energy electrons. Because many of these electrons traverse very short distances (a few nanometers) and deposit extremely high doses of radiation in the immediate vicinity of the decaying atoms, investigators have been interested in assessing in mammalian cells in vitro their radiotoxicity and other radiobiologic effects (morphological transformation, chromosomal aberrations, and mutagenesis, all of which have been used as surrogates for carcinogenesis). Studies have repeatedly shown the interdependence of the observed radiobiologic effects and intracellular or intranuclear localization of the low-energy electron emitter (*2–13*). For example, malignant transformation has been induced in BALB/3T3 mouse embryo fibroblasts by DNA-incorporated ^{125}I (*4*). Relative to incorporated ^{3}H or external x-rays, incorporated ^{125}I produces more transformants per surviving fraction. To our knowledge similar experiments have not been performed with intracellular, diagnostic radiopharmaceuticals.

The compound hexakis(2-methoxyisobutylisonitrile)-technetium(I) (^{99m}Tc-MIBI) is used routinely in myocardial perfusion imaging and has recently found a role in tumor imaging (*14*). We have assessed the oncogenic potential of ^{99m}Tc-Cardiolite using an in vitro cell transformation assay with C3H 10T1/2 mouse embryonic fibroblasts (*15–18*). Because the formulation of this radiopharmaceutical necessitates the addition to the Cardiolite kit (DuPont Merck Pharmaceutical Co., Billerica, MA) of sodium [^{99m}Tc]pertechnetate eluted from the ^{99}Mo–^{99m}Tc generator, the composition of the material being injected into the patient contains the radiopharmaceutical ^{99m}Tc-MIBI, any other components present in the Cardiolite kit (e.g., stannous chloride, tetrakis(2-methoxyisobutylisonitrile)copper(I) tetrafluoroborate), and trace amounts of impurities eluted from the generator (e.g., ^{99}Mo and alumina (*19*)). For this reason, 1-wk-old ^{99m}Tc-Cardiolite kit contents, referred to as decayed ^{99m}Tc-Cardiolite, served as the chemical control for

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these experiments (in this article we use the term ^{99m}Tc-Cardiolite, which means all components of the kit and those within the ^{99m}Tc-generator eluate, rather than the term ^{99m}Tc-MIBI, which suggests the pure radioactive compound). As a reference for the radiation action of ^{99m}Tc decay, we selected external irradiation with x-rays.

MATERIALS AND METHODS

Cells and Culture Conditions

C3H 10T1/2 (clone 8) cells were obtained from the American Type Culture Collection (Rockville, MD) at passage 12 or were kindly provided by Dr. John B. Little (Harvard School of Public Health) at passage 9. Cells were cultivated at 37°C in a humidified 95% air plus 5% CO_2 atmosphere in Basal Medium Eagle (GIBCO Laboratories, Gaithersburg, MD; no. 21010-046). Medium was supplemented with 10% defined fetal bovine serum (Hyclone, Logan, UT; no. SH30070, matched lots AGM7413 and AFC5040) and 25 μg/mL gentamicin (GIBCO, no. 15710-015). Cells were cultured in T75 flasks with an inoculation of 2×10^3 cells/mL. The doubling time was about 18 h. Confluency was reached on day 4 at $\sim 1.3 \times 10^6$ cells per flask. On day 3, cells were trypsinized (0.25% trypsin in 1 mmol/L EDTA·4 Na; GIBCO, no. 25200-072) and further cultivated or frozen in medium containing 20% serum and 10% dimethyl sulfoxide at 10^6 cells/mL per vial.

^{99m}Tc-Cardiolite and Decayed ^{99m}Tc-Cardiolite

^{99m}Tc-Cardiolite was obtained from the Joint Program in Nuclear Medicine Radiopharmacy, Harvard Medical School. ^{99m}Tc was eluted from a ^{99}Mo–^{99m}Tc generator (Technelite; DuPont Merck Pharmaceutical) every 24 h with 5–20 mL 0.9% NaCl. To ensure the consistency of pertechnetate samples, elutions for our experiments were always performed on the same day of the week. The ^{99m}Tc-Cardiolite solution was prepared by injecting various amounts of [^{99m}Tc]pertechnetate in 5 mL 0.9% NaCl into the Cardiolite kit, which contains tetrakis(2-methoxyisobutylisonitrile)copper(I) tetrafluoroborate (1.0 mg), stannous chloride dihydrate (0.075 mg), L-cysteine hydrochloride monohydrate (1.0 mg), sodium citrate dihydrate (2.6 mg), and mannitol (20 mg), and heating the mixture at 100°C for 1 h. The radioactive concentration of the ^{99m}Tc-Cardiolite (obtained from the radiopharmacy ~3 h after generator elution) varied between ~1.3 GBq/mL and ~7.4 GBq/mL. The sample was diluted in phosphate-buffered saline (PBS) to a final radioactive concentration of 0.037 GBq/mL and used either immediately (for exposure to ^{99m}Tc-Cardiolite) or after 1 wk of storage at −20°C (for exposure to decayed ^{99m}Tc-Cardiolite).

Exposure to ^{99m}Tc-Cardiolite, Decayed ^{99m}Tc-Cardiolite, and 20-Methylcholanthrene

For each experiment, 1 vial of 10^6 C3H 10T1/2 cells in passage 11–13 was used. Cells were thawed, plated in T75 flasks, and incubated at 37°C. On day 3, the cells were trypsinized, 10^5 cells were plated on a 100-mm Petri dish (P100), and reincubated at 37°C. The next day the medium was changed, and radioactive ^{99m}Tc-Cardiolite was added at ~50 to ~1200 kBq/mL. Extracellular radioactivity was measured immediately in triplicate 10-μL samples in a 1480 Wizard 3″ automatic γ counter (Wallac, Turku, Finland). The cells were then incubated at 37°C for 48 h, washed, and trypsinized, and survival and transformation assays were performed. To test the non-^{99m}Tc-related biologic effects of the ^{99m}Tc-Cardiolite solution, the experiment was repeated 1 wk later with the same solution of ^{99m}Tc-Cardiolite, which, by that time, consisted of decayed ^{99m}Tc-Cardiolite (the ^{99m}Tc atoms had decayed to ^{99}Tc). The same volumes of decayed ^{99m}Tc-Cardiolite in PBS were added to the cultured cells as had been used with ^{99m}Tc-Cardiolite 1 wk earlier.

As recommended by the International Agency for Research on Cancer (*20*), 20-methylcholanthrene ([MCA]; Sigma Chemical Co., St. Louis, MO; no. M-6501) was used as a positive control in the transformation assay. The compound was dissolved in acetone and diluted in medium (15 μg/mL), and the exposures were performed exactly as in the case of ^{99m}Tc- and decayed ^{99m}Tc-Cardiolite. The controls were treated with the same volume of acetone as required for treatment with MCA (final concentration of acetone in the medium, 0.24%).

Irradiation with X-Rays

External irradiation with x-rays was used as a reference for the radiation effects of exposure to ^{99m}Tc-Cardiolite. Fifty thousand cells were plated per T25 flask and, after 2 d of incubation at 37°C, the cells were irradiated at room temperature in an x-ray machine (Philips, Alpharetta, GA; 100 kV, no filtration, 0.52 Gy/min). Immediately after irradiation the cells were trypsinized and plated for survival and focus assays.

Survival and Transformation Assays

After treatment, cells were trypsinized, the concentration of the cells was adjusted for corresponding decreases in survival, and the cells were seeded into P100 plates (6 plates per dose for survival determination and 25 plates per dose for focus assay). In the survival assay, colonies were stained on day 10 and counted. The plating efficiencies of C3H 10T1/2 cells ranged from 18% to 28%, in accordance with the data of Chan and Little (*21*).

It is well established that focus formation in C3H 10T1/2 cell cultures treated with chemicals (*15,22*) and radiation (*16,23,24*) depends on the number of cell divisions after exposure (i.e., the number of cells seeded per plate in the focus assay). Our preliminary studies with ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite examined the influence of this factor. In the case of both radiation and chemical carcinogens, the number of foci induced decreases when the number of viable cells plated per 100-mm Petri dish increases from 50 to 1000 (*15,16*), but in the range of 200–500 viable cells per 100-mm dish, it is constant (*16,25*). In routine experiments we aimed at plating 300–400 viable cells per dish as is usual in most studies with radiation. Under these conditions cells multiply ~12-fold to reach confluency.

For the focus assay, the medium was changed twice a week for 2 wk and once a week thereafter. On day 41, the foci of transformed cells were stained, counted, and scored. In pilot experiments, the cells were washed with PBS, fixed with methanol for 10 min, and stained with 0.4% Giemsa solution (Sigma, no. GS-500) diluted 20-fold with PBS. In later experiments, we applied the method suggested by Balcer-Kubiczek et al. (*26*) and found that it gave better contrast between the foci and the surrounding monolayer. In this method the medium is removed and the cells are rinsed quickly with 4% formalin and fixed for 10 min with a freshly prepared mixture of 95% ethyl alcohol and 40% formaldehyde (7:1). After fixation, the cells are rinsed with water and stained for 3 min with crystal violet (6.5% of stock solution and 5% formalin in water; the stock solution of crystal violet is prepared by dissolving 1 g crystal violet in 65 mL glycerol at 60°C and combining with 65 mL methanol).

Foci of type 2 and type 3 were scored using morphological criteria described by Reznikoff et al. (*15*) and Terzaghi and Little (*16*). Foci of <2 mm were not scored (*20*). Because of the continuum of focus morphology, the intermediate foci (i.e., types

1–2 and 2–3) were scored conservatively. Mixed foci that had both type 2 and type 3 morphologies were scored progressively (*20*).

Analysis of Data

Transformation frequencies (TFs) per viable cell were calculated by the null method of Han and Elkind (*25*) in which focus induction is assumed to be an event having a Poisson distribution. After counting the foci, the fraction of plates without foci was obtained and used to calculate the mean number of foci per plate (λ):

$$\lambda = -\ln\left(\frac{\text{no. of dishes without foci}}{\text{total no. of dishes}}\right). \quad \text{Eq. 1}$$

The λ values are in good agreement with the number of foci per plate found by dividing the total number of foci by the total number of dishes per treatment (Table 1). This is indicative that foci induction has a Poisson distribution. Usage of this distribution allows the calculation of the error of TF from data obtained in a single experiment.

TF was calculated as the ratio of λ and the average number of viable cells per dish (product of the plating efficiency from the survival assay and the number of cells plated):

$$\text{TF} = \frac{\lambda}{\text{average no. of viable cells per dish}}. \quad \text{Eq. 2}$$

The SE of TF was obtained by dividing the SE of λ ($\sqrt{\lambda}$ divided by $\sqrt{}$total number of dishes) by the average number of viable cells (*27*).

Transformation curves were computer-fitted using Microcal software (Microcal Origin, version 4.10; Microcal Software Inc., Northampton, MA).

RESULTS

Morphology of Foci

When C3H 10T1/2 cells were exposed to ^{99m}Tc-Cardiolite or decayed ^{99m}Tc-Cardiolite and plated for focus formation, they reached confluence after ~12 doubling times (the number of cells per P100 dish was at that time about 1.3×10^6). Cells exposed to x-rays and MCA grew in the same way. On the lawn of confluent cells, the foci that emerged consisted of piles of cells that had lost contact inhibition. Figure 1 illustrates type 3 foci induced by the positive controls (MCA and x-rays) and by decayed ^{99m}Tc-Cardiolite. The photographs show that these foci are very similar to those described by Reznikoff et al. (*15*) for chemical carcinogens and by Terzaghi and Little (*16*) for x-irradiation.

Figure 2 shows a nontumorigenic focus (type 1) of untreated C3H 10T1/2 cells and foci induced by ^{99m}Tc-Cardiolite, classified as type 2 and type 3 (i.e., foci that are expected to be tumorigenic in 60%–100% of cases) (*15,16*). In Figure 2A, the morphology of the focus is typical for control C3H 10T1/2 cells: growing and packed cells but no circumference of swirling cells invading the contact-inhibited monolayer. Figure 2B represents a type 2 focus that was induced by ^{99m}Tc-Cardiolite. This focus is denser than type 1 and its edges invade the monolayer of contact-inhibited cells on which a small satellite focus is formed. A type 3 focus (Fig. 2C) shows typical crisscrossing and swirling of stellate cells invading the monolayer. In addition to the type 2 and type 3 morphologies presented in Figure 2, we observed epithelioid foci that were counted as type 2. In general, foci of cells treated with ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite exhibit a tendency to form islets of transformed cells in the vicinity of the main focus more frequently than foci of cells treated with x-rays or MCA.

TFs

The frequency of type 2 and type 3 foci induction after exposure to escalating concentrations of ^{99m}Tc-Cardiolite or decayed ^{99m}Tc-Cardiolite increases linearly when plotted on

TABLE 1

Transformation of C3H 10T1/2 Cells by Low Toxic Doses of X-Rays, ^{99m}Tc-Cardiolite, and Decayed ^{99m}Tc-Cardiolite

Treatment	Survival fraction ± SE*	Viable cells per dish	Total no. of dishes†	Dishes with foci (types 2 and 3)	Foci no.	Foci per dish × 10^{-2}	λ‡ ± SE × 10^{-2}	TF§ ± SE × 10^{-4}
None	1 ± 0.06	309	191 (8)	None	None	None	<0.52	<0.167
X-rays (Gy)								
0.5	0.89 ± 0.06	391	46 (2)	1	1	2.2	2.2 ± 2.2	0.56 ± 0.56
1.0	0.81 ± 0.05	326	47 (2)	3	3	6.4	6.6 ± 3.8	2.02 ± 1.16
2.0	0.66 ± 0.05	285	47 (2)	5	6	12.7	11.3 ± 4.9	3.95 ± 1.72
^{99m}Tc-Cardiolite¶ (kBq/mL)								
63 ± 7	1.03 ± 0.09	345	94 (4)	8	12	12.8	8.9 ± 4.4	2.58 ± 1.27
128 ± 10	0.99 ± 0.07	370	69 (3)	18	20	29.0	30.2 ± 8.1	8.15 ± 2.19
240	0.88 ± 0.01	269	25 (1)	7	7	28.0	32.8 ± 8.4	12.19 ± 3.14
303 ± 6	0.66 ± 0.03	253	69 (3)	27	28	40.6	38.4 ± 9.1	15.15 ± 3.61

*Average plating efficiency for control ± SE of mean is adjusted to survival fraction value of 1 ± SE.

†No. of experiments given in parentheses.

‡λ = −ln (dishes without foci/total no. of dishes); SE for λ is calculated as $\sqrt{}$(λ/total no. of dishes).

§TF is calculated as λ/average no. of viable cells per dish.

¶Or decayed ^{99m}Tc-Cardiolite volume equivalents. Results are pooled experiments (e.g., 63 kBq/mL [range, 56–83 kBq]; 128 kBq/mL [range, 107–139 kBq]; and 303 kBq/mL [range, 291–309 kBq]).

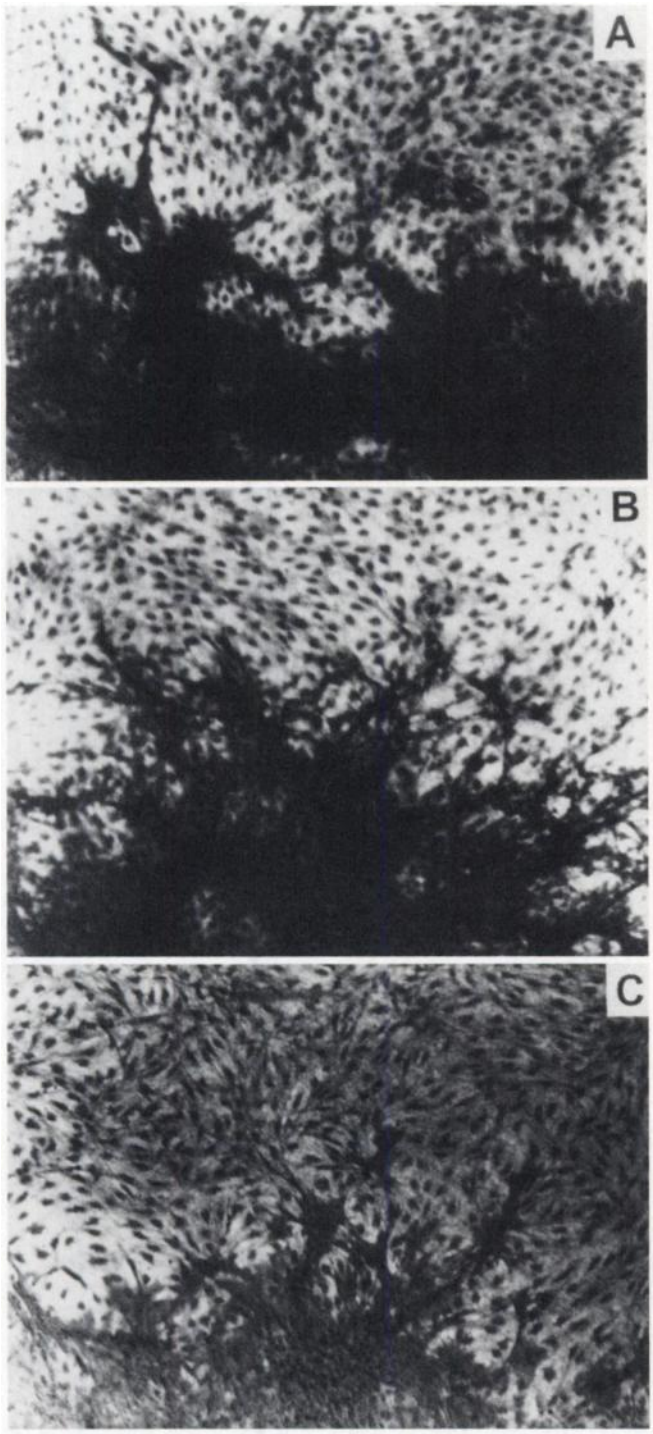

FIGURE 1. Edges of type 3 foci induced by 15 μg/mL MCA (A; crystal violet), 2-Gy x-rays (B; crystal violet), and 1110 kBq/mL (30 μCi/mL) volume equivalents decayed ^{99m}Tc-Cardiolite (C; Giemsa). Magnification, ×40.

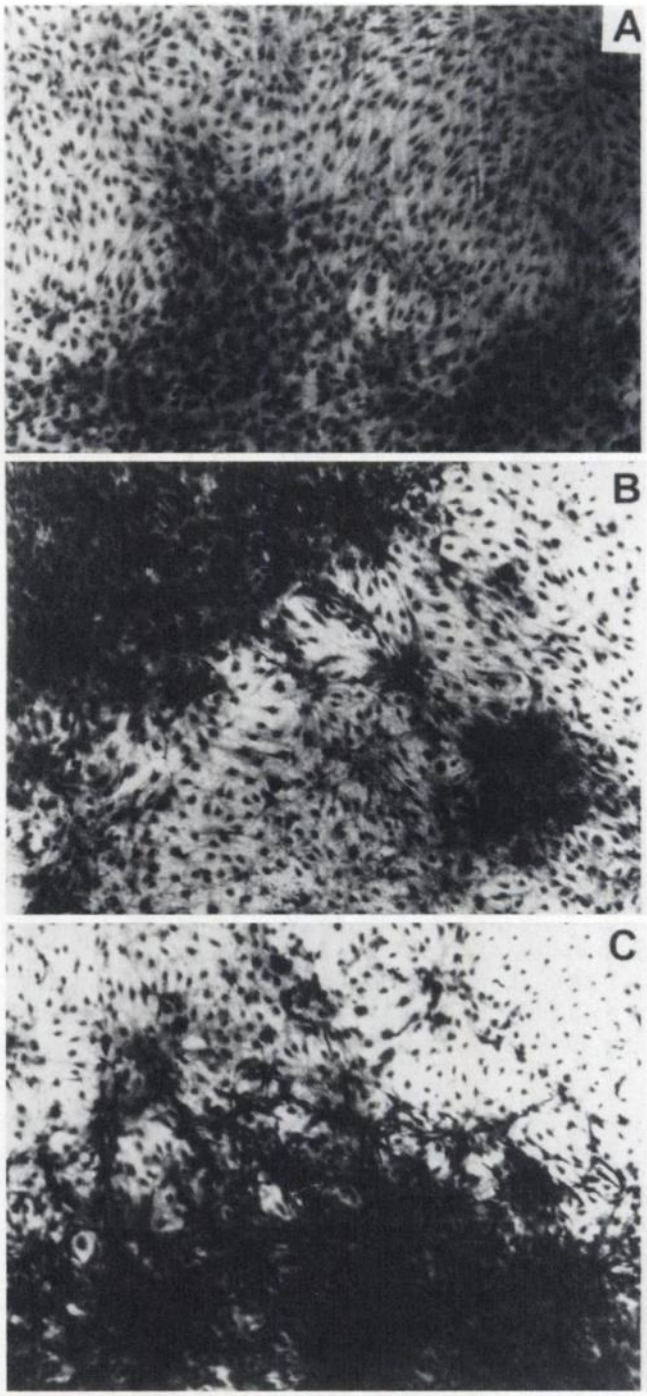

FIGURE 2. Circumferences of advanced type 1 focus of untreated control (A), type 2 focus induced by 292.3 kBq/mL (7.9 μCi/mL) ^{99m}Tc-Cardiolite (B), and type 3 focus induced by 292.3 kBq/mL (7.9 μCi/mL) ^{99m}Tc-Cardiolite (C). Crystal violet; magnification, ×40.

linear coordinates (Fig. 3A). The data points for both ^{99m}Tc- and decayed ^{99m}Tc-Cardiolite can be fitted with a single linear function ($r^2 = 0.98$). Presentation of the same data in semilogarithmic coordination (Fig. 3B) gives insight into TFs at low concentrations. Because the estimated frequency of spontaneous foci per viable cell in untreated control cells is $\leq 0.167 \times 10^{-4}$ (Table 1), the results indicate that the exposure of C3H 10T1/2 cells to ~37 Bq/mL (~1 μCi/mL) ^{99m}Tc-Cardiolite or its decayed volume equivalent leads to a substantial increase in the induction of transformants. This concentration is in the range initially obtained in a patient after the administration of 1.1 GBq (~30 mCi) ^{99m}Tc-Cardiolite. Inspection of focus morphology indicates that

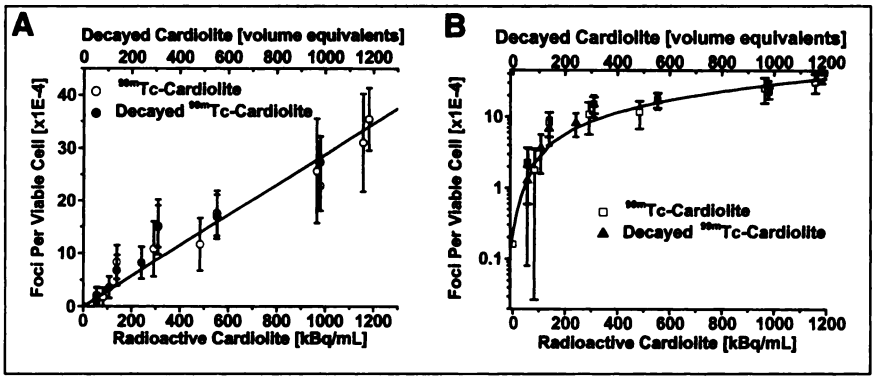

FIGURE 3. Transformation of C3H 10T1/2 cells by ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite. Data were obtained in 6 experiments. (A) Plot of frequency of induction vs. concentration of Cardiolite. Mean number of foci per viable cell and SEM were calculated from Poisson distribution. Because χ^2 analysis performed for different concentrations of ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite counterparts gives $P > 0.3$ in all cases, common linear fit was applied ($r^2 = 0.98$). (B) Semilogarithmic plot of same data as in (A) from which TF for untreated cells (Table 1) can be estimated. Note rapid increase in transformation induction after exposure to low concentrations of ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite.

~5% of all neoplastic foci induced by ^{99m}Tc- or decayed ^{99m}Tc-Cardiolite are type 3 (Table 2). Although the data show that the frequency of type 3 foci is somewhat higher after exposure to ^{99m}Tc-Cardiolite than to decayed ^{99m}Tc-Cardiolite (Table 2), the 2 groups are not significantly different at the 0.05 level (χ^2).

Comparison with X-Rays

The efficient induction of transformation by ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite was compared with the findings obtained after low-dose x-irradiation (Table 1 and Figures 4 and 5). In contrast with the linear induction of type 2 and type 3 foci by Tc-Cardiolite (Fig. 3), the TF as a function of x-ray dose has a linear–quadratic character (Fig. 4). In Figure 5 we compare the induction of transformants by low doses of Tc-Cardiolite and x-rays for which survival is high (100%–66%). We pooled the ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite transformation data from different experiments and averaged the respective extracellular concentrations of Tc-Cardiolite (Table 1). Whereas the TF after x-irradiation increases slowly, that after exposure to Tc-Cardiolite is very rapid even at the highest cell survival (>99%). In the case of the lowest x-ray dose studied (i.e., 0.5 Gy [survival, 89%]), a single type 3 focus was induced in 2 experiments (total, 46 dishes; foci/viable cell = 0.56×10^{-4}, Table 1). At the same survival level, Tc-Cardiolite induced 7 foci in 25 dishes (12.19×10^{-4} transformants per viable cell). Therefore, Tc-Cardiolite appears to be much more efficient than x-rays at the induction of transformation when cell survival is high.

TABLE 2
Type 2 and Type 3 Foci Induced by ^{99m}Tc-Cardiolite and Decayed ^{99m}Tc-Cardiolite

Dose (kBq/mL or volume equivalents)	Frequency			
	^{99m}Tc-Cardiolite		Decayed ^{99m}Tc-Cardiolite	
	Type 2	Type 3	Type 2	Type 3
56	2/3	1/3	3/3	0/3
139	8/8	0/8	6/6	0/6
309	10/12	2/12	12/12	0/12
553	14/16	2/16	13/13	0/13
979	20/20	0/20	21/22	1/22
Total	54/59*	5/59*†	55/56*	1/56*†

*Denominator = total no. of type 2 and type 3 foci.

†Values are not significantly different: $0.2 > P > 0.1$, based on χ^2 test.

DISCUSSION

This study was designed to test in vitro the neoplastic transformation capabilities of ^{99m}Tc-Cardiolite, a clinically useful myocardial perfusion agent (*28,29*) used also for

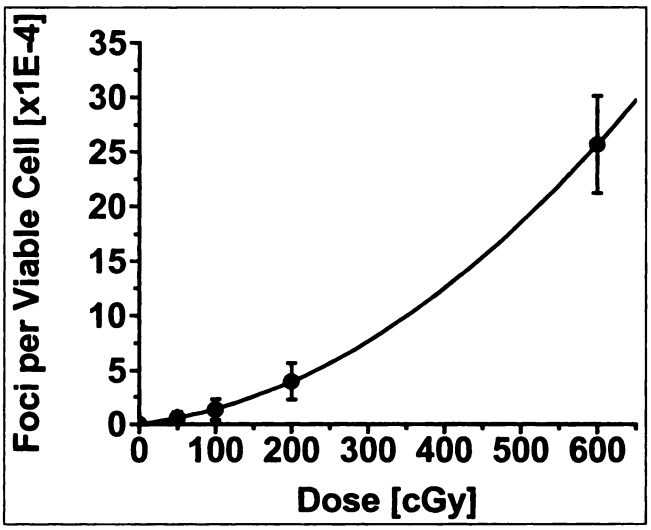

FIGURE 4. Transformation of C3H 10T1/2 cells by x-rays. Mean number of foci per viable cell and SEM were calculated from Poisson distribution and are fitted to second-order polynomial with $r^2 = 1.00$.

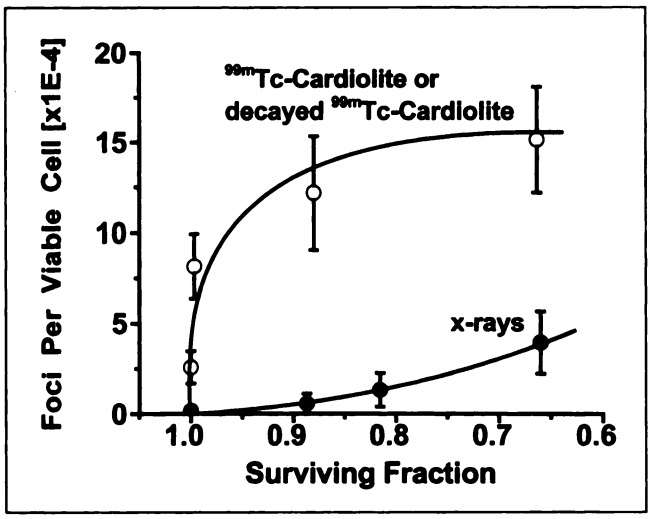

FIGURE 5. Comparison of transformation abilities of ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite with those of x-rays. Low-toxic-dose data from Figure 4 are replotted as function of cell survival. Curve for Cardiolite is fitted by eye. Data for x-rays are fitted to second-order polynomial with $r^2 = 0.99$.

tumor imaging (*30*). The radiopharmaceutical component of ^{99m}Tc-Cardiolite, ^{99m}Tc-MIBI, is a lipophilic cation that enters cells by diffusion across the cell membrane. It is localized in mitochondria (97%), and the mitochondrial transmembrane potential provides the net driving force for mitochondrial sequestration of this agent (*31*). As a result, its concentration in mitochondria may be ~1000-fold greater than the extracellular concentration. Several lines of evidence indicate that ^{99m}Tc-MIBI remains unmetabolized within the cell (*31–33*).

We adopted a C3H 10T1/2 mouse embryonic fibroblast system (*15*) that has been used extensively for examining the carcinogenic potential of various chemicals and types of radiation in vitro (*15,16,20,34*). In the transformation assay, morphologically transformed foci (type 2 and type 3; Fig. 2) are scored using defined criteria (*15,16,20*). Because the progeny of the cells derived from these foci are tumorigenic (60%–100%) when injected into mice (*15,16*), the assay is used to assess the oncogenic potential of various physical and chemical agents. In our studies, C3H 10T1/2 cells were exposed to ^{99m}Tc-Cardiolite or decayed ^{99m}Tc-Cardiolite for 48 h, the exposure time that is used in most studies of chemical carcinogens in vitro (*20*). This period covers 8 physical half-lives of ^{99m}Tc ($t_{1/2}$ = 6 h); thus, 99.6% of the ^{99m}Tc will have decayed during the period of exposure.

Figure 3 indicates that the exposure of C3H 10T1/2 cells to ^{99m}Tc-Cardiolite or decayed ^{99m}Tc-Cardiolite leads to a linear increase in the induction of type 2 and type 3 foci and that there is no statistically significant difference in the frequency of transformants between newly synthesized and decayed ^{99m}Tc-Cardiolite. These findings imply that the contribution of radiation from ^{99m}Tc decay to the formation of foci is negligible and suggest that a chemical mechanism probably underlies the induction of transformants. This might have been expected because many of the low-energy electrons emitted from the decay of ^{99m}Tc within mitochondria fail to reach the nucleus, a situation similar to that reported by Kassis et al. (*9*) in which the cytotoxic effects of the low-energy electron emitter ^{125}I decaying in mitochondria were found to be 80-fold less than those of intranuclear ^{125}I.

We also compared focus frequency as a function of cell survival after exposure to low cytotoxic doses (survival, ≥66%) of ^{99m}Tc-Cardiolite or decayed ^{99m}Tc-Cardiolite and x-rays. In the latter case, we used acute irradiation with a high dose rate that is well known to produce morphological transformation (Fig. 5 and Table 1). The dependence of TF on dose has a linear–quadratic character, and the numeric values are in accordance with those published for this cell line (*17,25,35*). This type of focus induction by x-rays contrasts with an abruptly rising curve for ^{99m}Tc-Cardiolite. Whereas only 0.56×10^{-4} foci were induced when the cells were exposed to 0.5 Gy of x-rays (survival, 89%), ^{99m}Tc-Cardiolite induced 12.19×10^{-4} transformants per viable cell at equivalent levels of survival (Table 1). The efficient transformation of C3H 10T1/2 cells by ^{99m}Tc-Cardiolite occurs while cell survival is minimally adversely affected.

It appears that the mechanism of morphological transformation of C3H 10T1/2 cells by ^{99m}Tc-Cardiolite and decayed ^{99m}Tc-Cardiolite is chemical in nature. This is not surprising because it is well known that ionizing radiation is a rather weak carcinogen in comparison with chemicals. However, the agent responsible for the induction of transformants is yet to be identified. For example, stannous chloride, 1 of the Cardiolite kit components, is known to be mutagenic in bacteria (*36*). The active intermediate in the synthesis, $[Cu(MIBI)_4]BF_4$, has been evaluated for genotoxic potential in a battery of 5 tests (Cardiolite [package insert]. Billerica, MA: DuPont Merck Pharmaceutical Co.; 1994), and no genotoxic activity was observed in the Ames, Chinese hamster ovary cell/hypoxanthine–guanine phosphoribosyl transferase, and sister-chromatid exchange tests in vitro. Furthermore, $[Cu(MIBI)_4]BF_4$ did not show genotoxic effects in the in vivo mouse micronucleus test at a dose that causes systemic and bone marrow toxicity. At cytotoxic concentrations, however, an increase in cells with chromosomal aberrations was observed in the in vitro human lymphocyte assay. These results do not exclude the possibility that compounds present in the kit may be responsible for the induction of transformation in our studies.

The acute toxicity of ^{99}Tc-MIBI (at concentrations 10^5 times higher than the tracer concentration used clinically) is ascribed to mitochondrial depolarization and uncoupling (*37*). $[Cu(MIBI)_4]BF_4$ is lipophilic and cationic and, therefore, probably also localizes in mitochondria. Loading mitochondria with $[^{99m}Tc(MIBI)_6]^+$, $[^{99}Tc(MIBI)_6]^+$, and $[Cu(MIBI)_4]^+$ molecules, even at small extracellular concentrations, might disturb the mitochondrial membrane potential, initiate excessive production of reactive oxygen species that damage mitochondrial and nuclear DNA, and cause

genomic instability leading to transformation many generations later. Impairment of mitochondrial oxidative metabolism has been implicated in genomic instability induced by ionizing radiations (*38,39*).

CONCLUSION

There is no increase in the induction of transformation with radioactive ^{99m}Tc-Cardiolite compared with decayed ^{99}Tc-Cardiolite, implying that low-energy electron irradiation from within the cytoplasm is a weak agent for cellular transformation. However, exposure of mammalian cells to low nontoxic concentrations of ^{99m}Tc-Cardiolite leads to the efficient transformation of these cells. Further studies are needed to identify the chemical(s) responsible for the induction of transformants and to determine whether the morphological transformants obtained in our in vitro studies are tumorigenic when injected into animals.

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