Cell Biology. In the article "ADP-ribosylation factor and phosphatidic acid levels in Golgi membranes during budding of coatomer-coated vesicles" by Mark Stamnes, Giampietro Schiavo, Gudrun Stenbeck, Thomas H. Söllner, and James E. Rothman, which appeared in number 23, November 10, 1998, of *Proc. Natl. Acad. Sci. USA* (**95,** 13676–13680), the authors wish to note the following correction. The label, described correctly in the legend to Fig. 1, incorrectly indicated that coatomer was included in stage 1 of the two-stage reactions. A corrected figure and its legend are shown below.

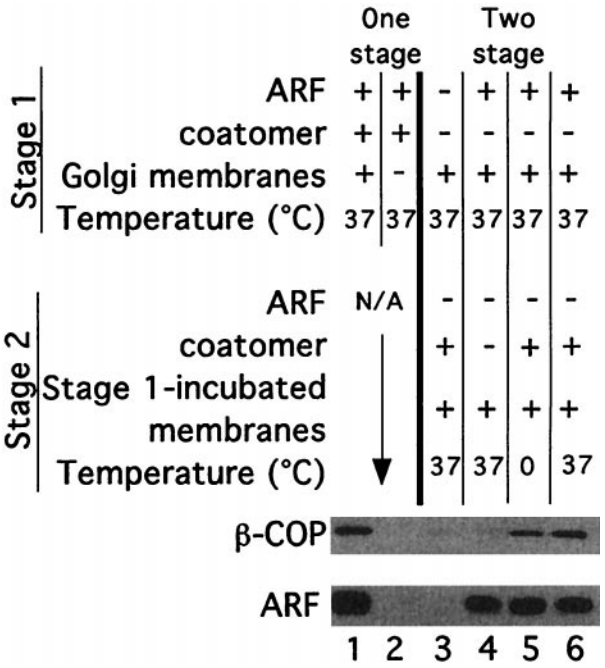

FIG. 1. ARF and coatomer binding in one- and two-stage reactions. The amount of membrane-bound ARF and coatomer (β-COP) determined by Western blot analysis of binding reactions. Lanes 1 and 2 show one-stage reactions in which ARF and coatomer were incubated together. Lanes 3–6 show the results from two-stage reactions in which the membranes were first incubated with ARF but not coatomer, reisolated, and then incubated in a second stage with coatomer but not ARF. As controls, membranes (lane 2) or ARF (lane 3) were excluded from stage 1, or coatomer (lane 4) was excluded from both stages. All incubations were carried out at 37°C except lane 5, which was carried out at 0°C.

Medical Sciences. In the article "Gadolinium(III) texaphyrin: A tumor selective radiation sensitizer that is detectable by MRI" by Stuart W. Young, Fan Qing, Anthony Harriman, Jonathan L. Sessler, William C. Dow, Tarak D. Mody, Gregory W. Hemmi, Yunpeng Hao, and Richard A. Miller, which appeared in number 13, June 25, 1996, of *Proc. Natl. Acad. Sci. USA* (**93,** 6610–6615), the following correction should be noted. It has come to our attention that the radiation sensitivity of the HT29 control cell line reported in Fig. 2 on page 6611 is inconsistent with that reported in the cited literature (33). Consequently, the *in vitro* HT29 radiation sensitization experiments have been repeated with Gd-tex^{2+} (compound **1**). Radiation enhancement comparable to our original findings was observed at doses between 8 and 20 Gy. The results indicate that the Gy scale reported on the *x*-axis of Fig. 2 is incorrect. We apologize for this error. The conclusions reached in the article remain unchanged.

Medical Sciences. In the article "Production of β-defensins by human airway epithelia" by Pradeep K. Singh, Hong Peng Jia, Kerry Wiles, Jay Hesselberth, Lide Liu, Barbara-Ann D. Conway, Everett P. Greenberg, Erika V. Valore, Michael J. Welsh, Tomas Ganz, Brian F. Tack, and Paul B. McCray, Jr., which appeared in number 25, December 8, 1998, of *Proc. Natl. Acad. Sci. USA* (**95,** 14961–14966), due to a printer's error, the following change should be noted: the symbol for Brian F. Tack should be ‡, to indicate that he is affiliated with the Department of Microbiology of the University of Iowa College of Medicine.

Medical Sciences. In the article "A multidrug resistance transporter from human MCF-7 breast cancer cells" by L. Austin Doyle, Weidong Yang, Lynne V. Abruzzo, Tammy Krogmann, Yongming Gao, Arun K. Rishi, and Douglas D. Ross, which appeared in number 26, December 22, 1998, of *Proc. Natl. Acad. Sci. USA* (**95,** 15665–15670), the following corrections should be noted. In the abstract and text, later analyses reveals that BCRP is a 655 amino acid peptide, not 663 amino acids as stated in the article. The first 8 amino acids displayed in Fig. 2*A* on page 15667 should be removed, making the initial sequence of the peptide MSSSNVEVFI. . . .

On page 15665 in the data deposition footnote, the GenBank database accession number for BCRP is incorrect. The correct accession number is AF098951.

On page 15670 in the "Note Added in Proof," the accession number for the human EST clone that was homologous to BCRP is incorrect. The correct number is HUEST157481.

Proc. Natl. Acad. Sci. USA
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Medical Sciences

Gadolinium(III) texaphyrin: A tumor selective radiation sensitizer that is detectable by MRI

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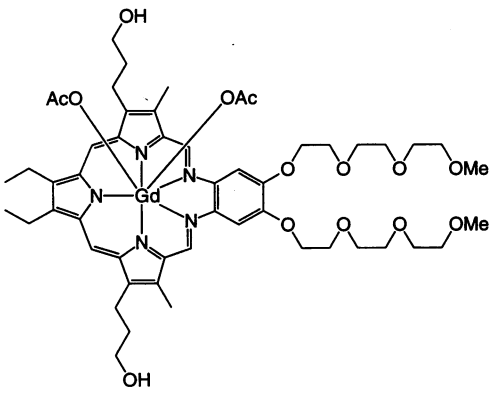
ABSTRACT **Gadolinium(III) texaphyrin (Gd-tex^{2+}) is representative of a new class of radiation sensitizers detectable by magnetic resonance imaging (MRI). This porphyrin-like complex has a high electron affinity [$E_{1/2}$ (red.) ≈ 0.08 V versus normal hydrogen electrode] and forms a long-lived π-radical cation upon exposure to hydrated electrons, reducing ketyl radicals, or superoxide ions. Consistent with these chemical findings, Gd-tex^{2+} was found to be an efficient radiation sensitizer in studies carried out with HT29 cells in *in vitro* as well as in *in vivo* single and multifraction irradiation studies with a murine mammary carcinoma model. Selective localization of Gd-tex^{2+} in tumors was confirmed by MRI scanning.**

Radiation therapy is a well established and important cancer treatment modality that is widely used (1, 2). Unfortunately, the therapeutic benefit of radiation therapy is limited by normal tissue tolerance and by tumor cell resistance to ionizing radiation (3, 4). Also limiting the efficacy of radiation therapy, often by a factor of 2.5–3 (5, 6), are the low levels of oxygen present in some portions of the tumor because the presence of oxygen may prolong the lifetime of cytotoxic free radicals generated upon exposure to ionizing radiation (7, 8). Previous attempts to overcome these limitations have included the use of radiation dose fractionation (1, 2, 7, 9) and the use of radiation sensitizers—drugs that potentiate the efficacy of the delivered radiation (7, 8, 10–13). Agents that have been explored extensively in this latter context include the halogenated pyrimidines (14–18) and hypoxic cell sensitizers (e.g., nitroimidazoles) (19–21). However, to date, these compounds have had some associated toxicity and do not adequately sensitize the entire tumor cell population (8). They also lack the preferential localization in tumors required to increase the therapeutic index (14), although the radiation therapy itself can to some extent be localized. With halogenated pyrimidines, a mechanistic dependence on incorporation of the drug into replicating DNA also has limited efficacy since many tumors contain a low fraction of cells in S phase (22, 23). There remains a need for improved radiation sensitizers. Ideally, these should (*i*) potentiate the activity of the administered radiation in the tumor but not in the surrounding tissues, (*ii*) operate via a mechanism that is active against oxygenated and hypoxic cells and is independent of DNA incorporation, and (*iii*) have low inherent toxicity. We have developed a new radiation sensitizer [i.e., gadolinium(III) texaphyrin (Gd-tex^{2+})] that, due to its novel mechanism of action, and tumor-selective localizing ability, meets these criteria. An additional benefit of this radiation sensitizer that has not been available with previous sensitizers is that it is detectable *in vivo* by magnetic resonance imaging (MRI) methods (24, 25). Monitoring the selective accumulation of gadolinium(III) texaphyrin in neoplasms by MRI enables the possibility of treatment planning and subsequent monitoring of the response



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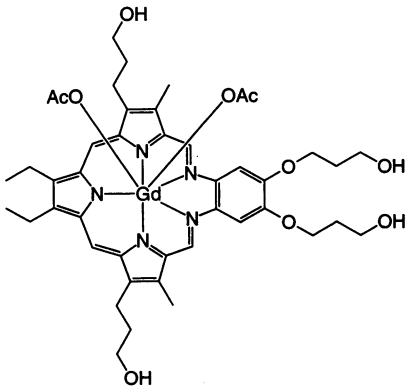

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of cancers to the radiation therapy. Gadolinium(III) texaphyrin is representative of a new class of compounds known as the texaphyrins (e.g., structures **1** and **2**) (25–27).

Texaphyrins are large planar porphyrin-like macrocycles that are capable of coordinating a range of relatively large cations, including Gd(III) and other members of the trivalent lanthanide series (26, 27). In general, the complexes formed are stable and of a 1:1 metal-to-ligand stoichiometry (27). However, the complexes are also easily reduced [E (red.) ≈ 0.08 V versus normal hydrogen electrode for both compounds **1** and **2**] and this facile reduction process, coupled with a demonstrated ability to localize selectively in certain animal tumor models (24), led to a consideration that these species

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Abbreviation: Gd-tex^{2+}, gadolinium(III) texaphyrin.

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**The redox potential of Gd-tex^{2+} is well above the upper threshold proposed for electron-affinic hypoxic cell sensitizers.

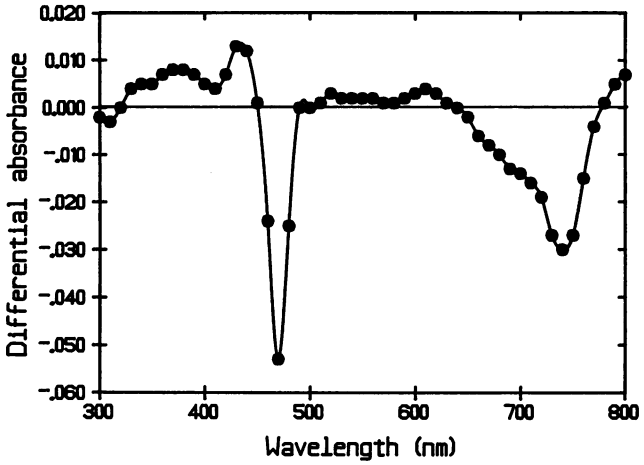

FIG. 1. Differential absorption spectrum of the π-radical cation obtained from complex **2** via pulse radiolytic reduction. The spectrum was recorded 25 μs after the pulse. Identical results were obtained using complex **1**.

could function as effective radiation sensitizers (28).** Our hypothesis was that, like molecular oxygen, the easy-to-reduce metallotexaphyrins would be able to "capture" hydrated electrons (e^-_{aq}) and thus increase the concentration of hydroxyl radicals available after exposure to a given dose of ionizing radiation. In addition, it was recognized that certain paramagnetic texaphyrin complexes, including the Gd(III)-containing species **1** and **2**, are detectable by MRI (24) and that this ready visualization would provide a means for determining directly the biolocalization properties (both temporal and spatial) of this new class of putative radiation sensitizer.

MATERIALS AND METHODS

Synthesis. Gadolinium texaphyrins **1** and **2** were prepared in accord with the procedure described earlier (26).

Cyclic Voltammetry. The quoted redox potentials were determined by cyclic voltammetry in 2 mM aqueous phosphate solution. Under these conditions, the one-electron reduction potential of both compounds **1** and **2** is independent of pH ($4 < pH < 10$). However, the electrode process is quasi-reversible, with at least two other, more cathodic, reduction waves being apparent in the voltammograms.

Pulse Radiolytic Studies. All pulse radiolysis studies were made using a 4-MeV van der Graaff electron beam accelerator (Center For Fast Kinetics Research, University of Texas; 1 eV = 1.602×10^{-19} J). Solutions of the gadolinium(III) texaphyrin complex in question (1×10^{-4} M) were made up in 2 mM sodium phosphate (pH 7) and studied in the presence of 0.1 M 2-propanol. The solutions were purged thoroughly with oxygen-free N_2 prior to irradiation and a fresh aliquot was used for each pulse (100-ns duration). The course of reaction (if any) was followed by transient absorption spectroscopy. Differential absorption spectra were recorded point-by-point with three individual shots being averaged at each time base. Data analysis was made by computer nonlinear least-squares iteration. Dosimetry was made with the thiocyanate dosimeter

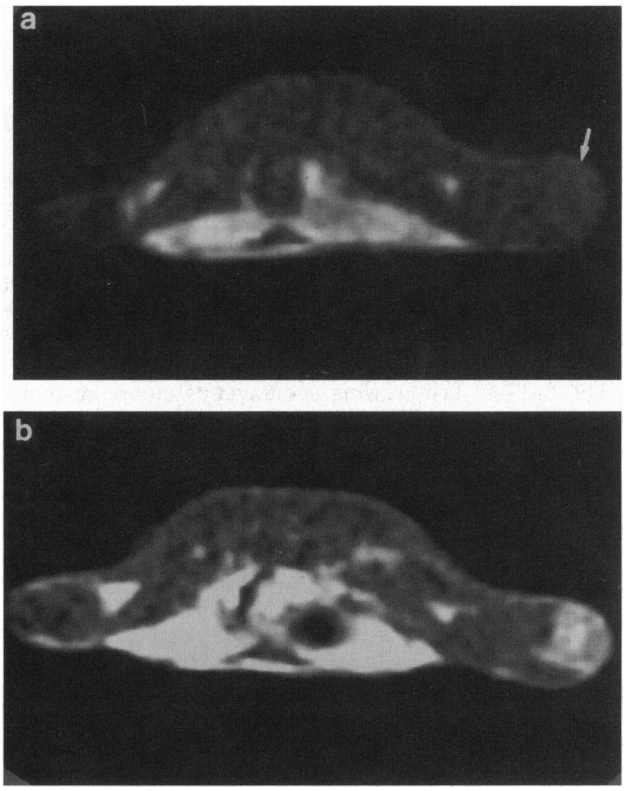

FIG. 3. (*a*) Precontrast: Axial MRI scan obtained through an SMT-F tumor in the right leg (arrow) of a DBA/2N mouse. Pulsing sequence: 0.5 T, TR/TE 350/15/FR (3-mm slice thickness 256 × 256, 2 NEX, variable band width). (*b*) MRI at same level as *a* 10 min after the injection of Gd-tex^{2+} (40 μmol/kg) as a 2 mM solution in 5% aqueous mannitol. Pulsing sequence: 0.5 T, TR/TE 350/15/FR (3-mm slice thickness 256 × 256, 2 NEX, variable band width).

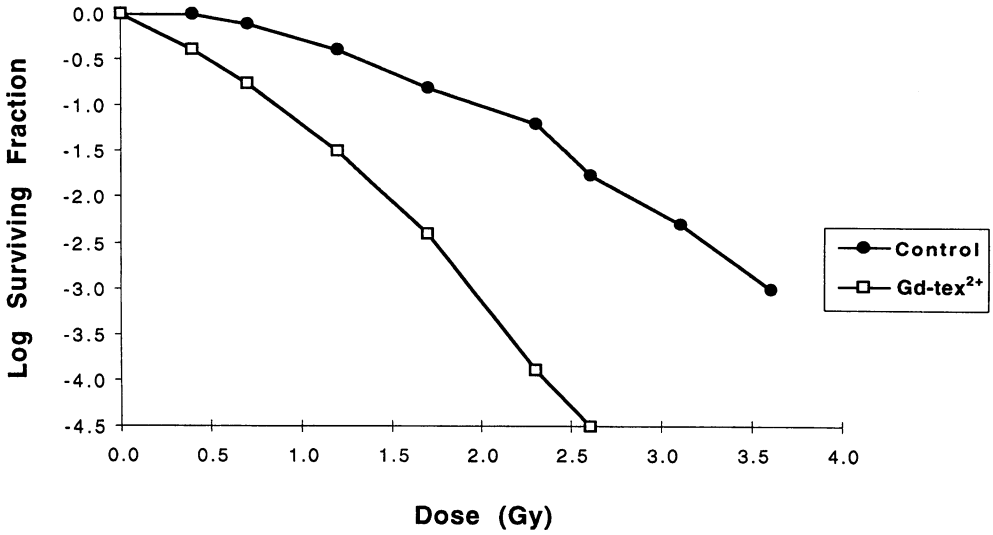

FIG. 2. Effect of radiolysis on cell survival for HT29 cells. Control experiments were carried out with HT29 cells not exposed to Gd-tex^{2+} but irradiated under identical conditions (all standard deviations were less than ±10%).

(29, 30). For kinetic analyses, the concentration of the material under investigation was varied systematically and the rate of formation (or decay) of the relevant transient species was measured at each concentration.

Cells and Tumor Models. Murine leukemia L1210 and HT29 human colon cancer cells were obtained from the American Type Culture Collection. L1210 cells, a suspension cell line, were grown in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum and gentamycin (5 μg/ml). HT29 cells, an adherent cell line, were maintained in minimal essential medium (MEM) (GIBCO/BRL) supplemented with 10% fetal bovine serum and gentamycin (5 μg/ml). *In vitro* studies were performed in growth medium with 25 mM Hepes (pH 7.2).

The SMT-F, fast-growing spontaneous mouse mammary tumor, and the EMT-6 tumor cell line, murine mammary sarcoma, were obtained from J. Martin Brown (Stanford School of Medicine). The SMT-F tumors were maintained in DBA/2N mice according to Pavelic *et al.* (31). EMT-6 cells are syngeneic to BALB/c mice and were propagated according to the protocol of Rockwell *et al.* (32). Female mice weighing 18–22 g, 10–12 weeks old, were obtained from Simonsen Laboratories (Gilroy, CA). The tumor cells (5 to 7 × 10^5 cells) were implanted into the right hind gastrocnemius of recipient mice. The tumor-bearing animals were studied when the tumor size was 40–70 mm^2 (5–7 days after implantation). Tumor size was based on two orthogonal cross-sectional diameter measurements from the tumor-bearing leg and were measured biweekly.

Radiation Sensitization of Cancer Cells *in vitro*. The *in vitro* radiosensitization experiments were adapted from a procedure utilized by Miller *et al.* (33) and involved both L1210 and HT29 cells. Briefly, for the HT29 adherent cell line, the cells were incubated for 24 h with a fixed amount of gadolinium(III) texaphyrin (either compounds **1** or **2**; 10^{-7} M to 10^{-2} M), washed thoroughly with PBS, trypsinized, and resuspended in fresh medium (5 ml) at a density of 2 × 10^5 cells per ml. The cells were exposed to radiolysis for fixed times. Irradiation was made with doses of 250-kV x-rays generated by a Philips TR 250 orthovoltage x-ray machine at a dose rate of 1.25 Gy/min. Three dishes were plated for each dose, which were left to incubate for 10 days at 37°C. The numbers of surviving colonies (>50 individual cells) were counted after staining with crystal violet and compared with the value obtained for unirradiated cells to estimate the sensitizer enhancement ratio.

L1210 cells were treated in the same manner as above with some experimental modifications to allow for differences between adherent versus suspension cell lines and also growth kinetics. Briefly, the L1210 cells were suspended at a density of 5 × 10^5 cells per ml (5 ml) prior to exposure to radiation. The cells were then resuspended at a density of 1 × 10^5 cells per dish and incubated for 7 days. Cell viability was assessed using the trypan blue exclusion method. Radiosensitization efficacy was expressed as the amount of cell killing achieved without and with a particular concentration of sensitizer after exposure to 2 Gy. The actual values were extrapolated from cell survival versus dose curves using a nonlinear least-squares iterative procedure to fit the data points.

MRI Studies. To assess the biodistribution of compound **1** MRI scans in SMT-F mice were performed. A solution of Gd-tex^{2+} (complex **1**, 2 μmol/ml in sterile 5% aqueous mannitol) was administered i.v. via the tail vein at a dose of 40 μmol/kg. Axial MRI scans of the SMT-F tumors were obtained at 0.5 T, with the mouse (n = 4) in the prone position, using a spin-echo T1 weighted pulsing sequence (TR/TE, 350/15). MRI scans were performed before the dose and at 10 min and 1, 2, 3, 4, 5, 12, and 24 h after Gd-tex^{2+} i.v. injection. Contrast enhancement (CE) was determined by obtaining the signal intensity (SI) readings from a cursor placed over the SMT-F tumors by using the following formula: CE = (SI post − SI pre/SI pre) × 100.

Radiation Sensitization *in vivo*. After pilot studies,†† the following protocol was initiated: Gd-tex^{2+} at a dose of 40 μmol/kg was administered i.v. to SMT-F-bearing mice. The mannitol solution was administered i.v. to SMT-F bearing mice

††Pilot studies were conducted using SMT-F-tumor-bearing animals as follows: Animals were studied after a single dose of 10–50 Gy of radiation, at 30 min to 24 h after Gd-tex^{2+}, and after Gd-tex^{2+} at 40 μmol/kg i.v. (50% of the LD_{10} of 80 μmol/kg). Irradiation at 1 h or earlier produced morbidity and mortality and the optimal time window of irradiation appeared to be between 2 and 5 h after injection of Gd-tex^{2+} at 40 μmol/kg i.v. In addition, the maximal beneficial effect of Gd-tex appeared to be associated with a single 30-Gy dose of radiation.

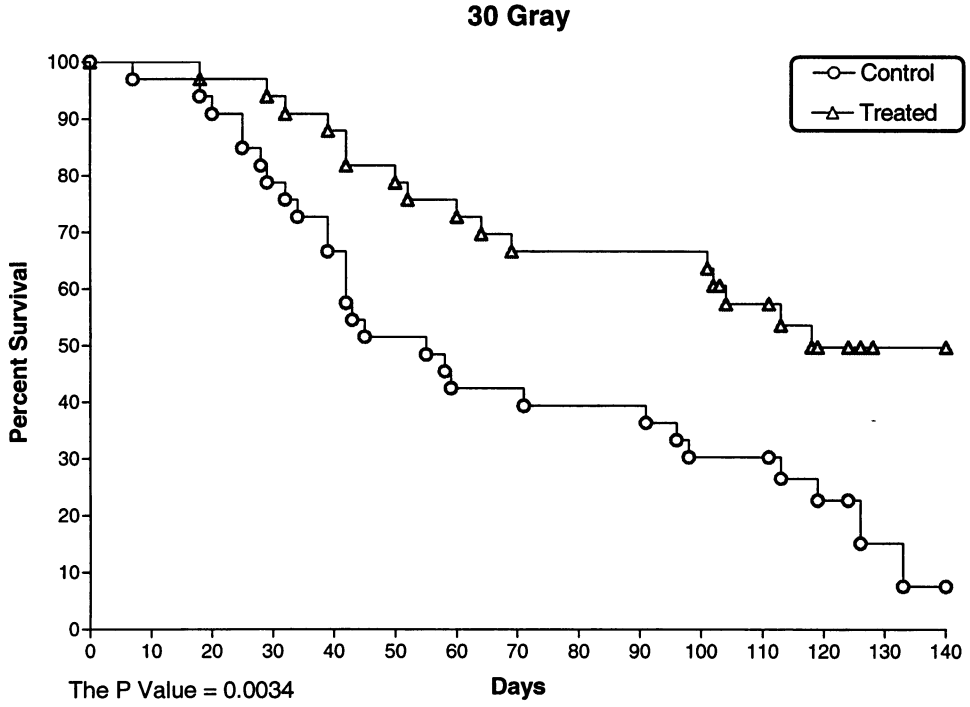

FIG. 4. Kaplan–Meier survival curves of DBA/2N mice with SMT-F neoplasms after 30-Gy single dose irradiation and i.v. administration of Gd-tex^{2+} at 40 μmol/kg (treated) and a matched set of control animals treated with 30-Gy of irradiation only. Also note that 16 of 33 animals were cured (no evidence of disease). Note the significant difference in survival (P = 0.0034) for those animals receiving Gd-tex^{2+} (n = 66).

and they served as a control group (n = 33 mice per group). The test group was divided and irradiated at 2 and 5 h after drug administration (single fraction of 30 Gy). A special leg jig (with lead shield) was used during the treatment. Each mouse was positioned prone inside a jig individually such that only the right leg was exposed to the x-ray. The tumor response and animal survival was evaluated for 140 days.

EMT6 tumors were found to be more resistant to radiation than the SMT-F tumors so the EMT6 tumor line became the tumor line used subsequently in the multidose fractionation studies. The sensitizer was equally effective when radiation was given at 2–5 h after Gd-tex^{2+} i.v., so radiation was given at 2 h in these studies. Gadolinium texaphyrin complex **1** (2 μmol/ml in 5% mannitol) or 5% mannitol was administered i.v. for five consecutive days to EMT6-bearing mice in the designated test and control groups, respectively (n = 6 mice per group). Two hours after each i.v. injection, x-ray treatment (five fractions of 1, 2, or 4 Gy) was administered. The study duration was 45 days and consisted of 78 mice. Mice in the 4-Gy protocol were treated in two study groups [i.e., (*i*) control and 5 and 20 μmol/kg and (*ii*) control and 40 μmol/kg].

In all of the studies described above, an additional set of animals was injected with comparable amounts of gadolinium texaphyrin i.v. in the absence of radiation. No difference in tumor growth or host survival was found between the control animals and those animals which received gadolinium texaphyrin i.v. in the absence of radiation.

RESULTS AND DISCUSSION

Pulse Radiolytic Studies. Initial tests of the MRI-detectable gadolinium(III) texaphyrin complexes **1** and **2** ("Gd-tex^{2+}") as a radiation sensitizer utilized pulse radiolysis (29, 30). Short bursts of ionizing radiation (100-ns duration at 4 MeV) were delivered into aqueous solutions of these complexes and the subsequent reactions monitored by transient absorption spectroscopy. The conditions were chosen so as to favor a reducing environment. The initial texaphyrin solutions (aqueous, pH 7, 1×10^{-4} M) were saturated with nitrogen after the addition of 2-propanol (0.1 M).‡‡ Under these conditions, hydroxyl radicals formed in the primary radiolysis event rapidly abstract the tertiary hydrogen atom from 2-propanol, forming highly reducing ketyl radicals (Eq. **1**).

$$OH\cdot + (CH_3)_2CHOH \rightarrow H_2O + (CH_3)_2COH\cdot \quad [1]$$

Both this ketyl radical and the hydrated electrons (also present in the medium) reduce the Gd-tex^{2+} complexes **1** and **2** via one-electron processes (Eqs. **2** and **3**):

$$\text{Gd-tex}^{2+} + e^-_{aq} \rightarrow \text{Gd-tex}^{+\cdot} \quad [2]$$

$$\text{Gd-tex}^{2+} + (CH_3)_2COH\cdot \rightarrow \text{Gd-tex}^{+\cdot} + (CH_3)_2CO + H^+ \quad [3]$$

It was further shown that superoxide ions reduced Gd-tex^{2+}.

$$O_2 + e^-_{aq} \rightarrow O_2^{-\cdot}$$

$$O_2^{-\cdot} + \text{Gd-tex}^{2+} \rightarrow O_2 + \text{Gd-tex}^{+\cdot}$$

Thus, all of the reducing equivalents can be utilized to reduce Gd-tex^{2+} to Gd-tex$^{+\cdot}$, regardless of the reaction conditions.§§ The resultant π-radical cation of Gd-tex^{2+} (Gd-tex$^{+\cdot}$), which is readily detected by monitoring the appropriate absorption spectral changes (Fig. 1), was found to decay over several hundred microseconds. This decay process, studied most closely in the case of complex **2**, does not result in restoration of Gd-tex^{2+}. However, the rate increases with decreasing pH and is thus attributed to protonation of the initially formed π-radical cation (Eq. **4**):

$$\text{Gd-tex}^{+\cdot} + H^+ \rightarrow \text{Gd-tex(H)}^{2+\cdot} \quad [4]$$

The resulting protonated radical (Gd-tex(H)$^{2+\cdot}$) decays very slowly by complex reactions that do not restore the original Gd-tex^{2+} complex (the life time is on the order of 30 s and is unaffected by the presence of oxygen).

In view of the high intrinsic stability of the Gd(III) oxidation state, Gd-tex$^{+\cdot}$ and Gd-tex(H)$^{2+\cdot}$ are presumed to be π-radical cations that have the reducing equivalent stored on the macrocyclic ring and not on the metal center. While it is clear that other factors, such as reduction of glutathione levels or inhibition of DNA repair processes might be serving to potentiate a putative sensitization effect *in vitro* or *in vivo*, the promise inherent in this seemingly unique mechanism of action prompted us to test Gd-tex^{2+} under more clinically relevant conditions. Summaries of these studies follow.

Radiation Sensitization of Cancer Cells *in Vitro*. Both compounds **1** and **2** were tested for their *in vitro* radiation sensitizing ability. These studies indicated that texaphyrins (compounds **1** and **2**) are effective radiosensitizers for L1210 cells under aerobic conditions. In fact, for both complexes **1** and **2**, the amount of cell killing [(L1210 + 2 Gy)/(L1210 + 2 Gy + Gd-tex^{2+})] was found to increase progressively with increasing concentration of drug beginning at 10^{-5} M and reaching a maximum value of 2.2 ± 0.03 at 10^{-3} M and above. Of course intracellular concentration of Gd-tex^{2+} may be and probably is different than the extracellular concentration. Gd-tex^{2+} (complex **1**) was also found to be an effective radiation sensitizer for HT29, a human colon cancer cell line (Fig. 2). Each data point is the mean of three separate runs (SD less than ±10%). The sensitization enhancement ratio for this experiment is 1.92 and is derived by comparing the radiation dose needed to kill 95% of the exposed cells in the absence and presence of sensitizer. These studies provided an indication that systems such as compounds **1** and **2** could function as effective *in vivo* radiosensitizers. The results of these studies were found to be independent of the specific complex employed (i.e., compounds **1** or **2**), suggesting that the sensitizing effect of Gd-tex^{2+} derives from the basic macrocyclic structure and its properties (e.g., ease of reduction) rather than a judicious choice of exocyclic substituents.

MRI and Radiation Sensitization in Animals. Based on the positive results obtained in cell culture, MRI and radiation sensitization studies were performed in DBA/2N mice with SMT-F tumors or EMT6 tumors in BALB/c mice (as noted both are transplantable mouse mammary carcinomas).

MRI scans after i.v. administration of Gd-tex^{2+} (2 μmol/ml in sterile 5% aqueous mannitol) revealed contrast enhancement of the SMT-F neoplasms. Maximal contrast enhancement was noted immediately after injection but at least 30% enhancement of the tumor (as opposed to surrounding tissue) was observed up to 5 h (Fig. 3) after injection. The observed

‡‡In our discussion, we treat the gadolinium(III) texaphyrin complexes **1** and **2** as monomers since, although these species are aggregated at higher concentrations (≥0.2 and ≥0.1 mM in the case of compounds **1** and **2**, respectively), we have not found experimental evidence to suggest that adjacent molecules affect the radiation chemistry to any significant extent. Adding mannitol (5% by weight) serves to reduce the degree of aggregation; under these conditions, critical aggregation concentrations of 2.6 and 1.0 mM are recorded for compounds **1** and **2**, respectively.

§§Additional pulse radiolysis experiments showed that the Gd-tex$^{+\cdot}$ radical can also be formed by reduction of Gd-tex^{2+} with carbon dioxide π-radical anion ($CO_2^{\cdot}$), and the carbon-centered radicals formed by hydrogen abstraction from ethanol and methanol; protonation in accord with Eq. **4** yields the Gd-tex(H)$^{2+\cdot}$ species. This protonated radical is also obtained directly via the reaction of hydrogen atoms and Gd-tex^{2+}.

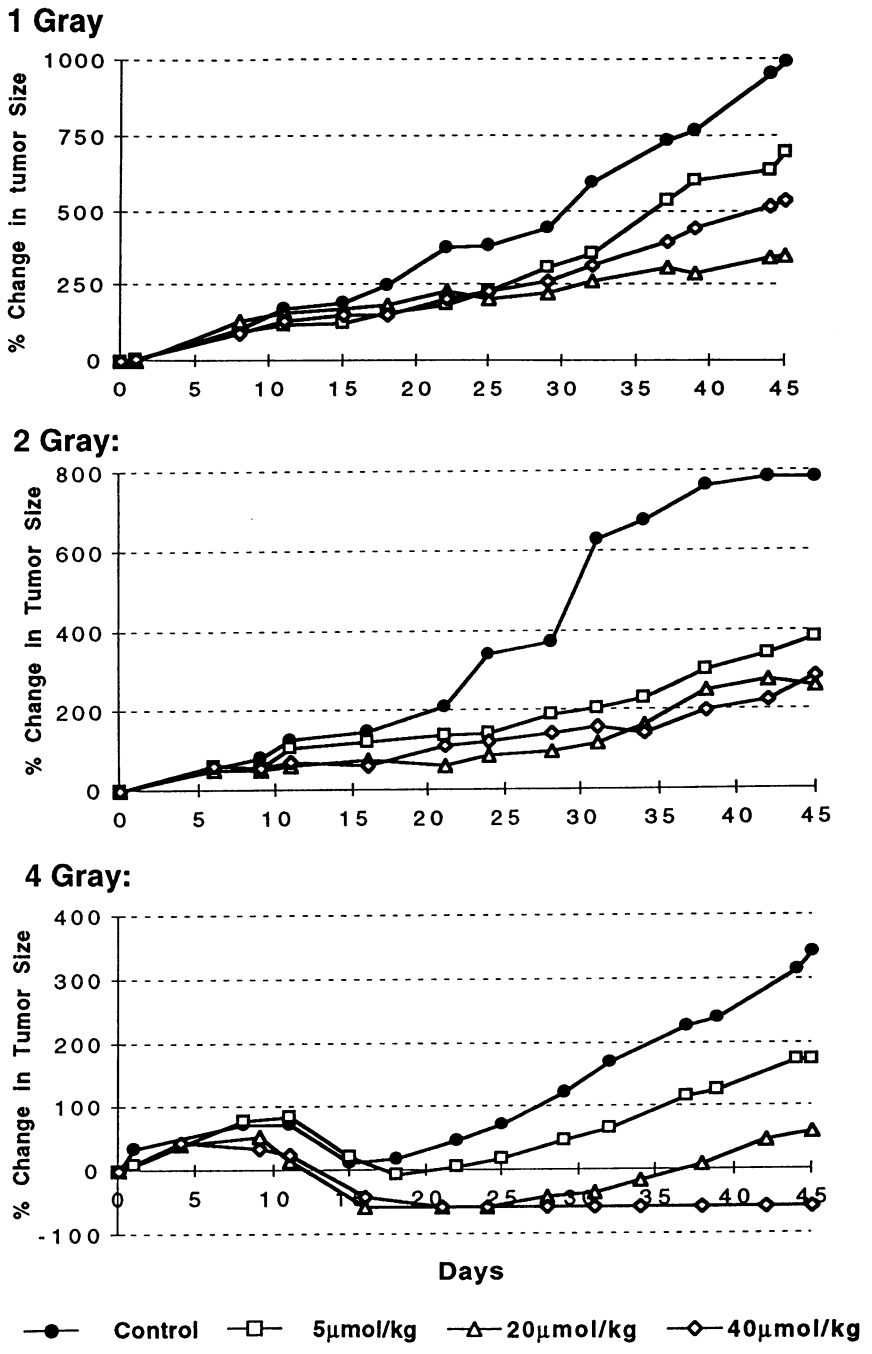

FIG. 5. Percent change in tumor size after i.v. injection of Gd-tex^{2+} at 5, 20, or 40 μmol/kg into BALB/c mice with EMT6 neoplasms irradiated for five consecutive days with either 1, 2, or 4 Gy per fraction.

augmentations in tumor signal intensity (after 40 μmol/kg) were as follows: 94%, 10 min; 79%, 1 h; 55%, 2 h; 44%, 3 h; 39%, 4 h; 32%, 5 h; 9%, 12 h; 7%, 24 h.

Administration of Gd-tex^{2+} (40 μmol/kg i.v.) prior to a single fraction of radiation provided a significant improvement in survival in SMT-F-bearing DBA/2N mice compared with animals receiving 30-Gy radiation alone ($P = 0.0034$) (Fig. 4). For animals receiving irradiation at both 2 h ($n = 32$) and 5 h ($n = 34$) after administration of gadolinium texaphyrin, significant therapeutic effects on tumor size were observed ($P = 0.0439$ and 0.0317, respectively). There were no significant differences in survival between the groups receiving Gd-tex^{2+} at 2 h versus 5 h prior to 30 Gy of irradiation ($P > 0.3495$, unpaired t test).

A significant radiation sensitization effect was shown in the five consecutive day multifraction studies with BALB/C mice bearing EMT6 neoplasms in the right leg that were injected with compound **1** (5, 20, or 40 μmol/kg) or control solutions of 5% aqueous mannitol 2 h prior to 1, 2, or 4 Gy of radiation therapy (Fig. 5). Even after 1 Gy of radiation for 5 days, there was a significant difference between the groups receiving 20 and 40 μmol/kg and controls ($P = 0.003$ and $P = 0.005$, respectively), although the group receiving 5 μmol/kg was not significantly different than controls ($P = 0.105$). Similarly, for all test groups in the 2- and 4-Gy study, EMT6 tumors were at least 50% smaller than control tumors at 45 days, and in the 4-Gy studies, there was a clear drug dose–response relationship for tumor size change (Fig. 5). By using a modification of methods to evaluate radiation induced toxicity to normal tissues, we evaluated short-term skin erythema (34) and long-term leg contracture (35) after treatment with the gadolinium complex **1** in conjunction with radiation. These results indicated that there was no enhanced radiosensitization of normal tissues when Gd-tex^{2+} was present.

Consistent with chemical findings from pulse radiolysis and cyclic voltammetry, the gadolinium(III) texaphyrin complex **1**

was found to be a very efficient radiation sensitizer, as judged from experiments involving tumor cells *in vitro* and SMT-F and EMT6 neoplasms in mice. Selective localization of Gd-tex^{2+} in tumors was confirmed through the MRI contrast enhancement afforded by the paramagnetic nature of the agent. The imaging studies serve to highlight the possibility of using the MRI contrast enhancement properties of this particular texaphyrin complex to facilitate treatment planning and response monitoring in the context of x-ray therapy of cancer.

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