Application of a Macromolecular Contrast Agent for Detection of Alterations of Tumor Vessel Permeability Induced by Radiation

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ABSTRACT

Permeability of tumor vasculature can be a major barrier to successful drug delivery, particularly for high molecular weight agents such as monoclonal antibodies and their diagnostic or therapeutic conjugates. In this study, changes in permeability of SCCVII tumor vessels after radiation treatment were evaluated by dynamic magnetic resonance imaging as a function of time after irradiation using a generation-8 polyamidoamine dendrimer (G8-Gd-D)-based magnetic resonance imaging contrast agent shown previously to be confined to tumor blood vessels. Tumor irradiation consisted of either single doses (2–15 Gy) or various daily fractionated doses (5 days). A single radiation dose of 15 Gy resulted in significant transient image enhancement of the tumor tissue with a maximum occurring between 7 and 24 hours after radiation treatment. No observable enhancement was recorded for fractionated radiation doses. Use of dynamic magnetic resonance imaging coupled with G8-Gd-D provides an exquisite methodology capable of defining the timing of enhanced permeability of macromolecules in tumors after irradiation. Such information might be applied to optimize the efficacy of subsequent or concurrent therapies including radiolabeled antibodies or other anticancer agents in combination with external beam therapies.

INTRODUCTION

The composition of tumor tissue vasculature is strikingly different as compared with normal tissue (1). Tumor vessels are typically irregular and tortuous; exhibit various branching patterns including shunts and blind ends; and are deficient of smooth muscles, nerves, and basement membranes; and display abnormal blood flow patterns (2–5). In addition, tumor vessels are often “leaky” resulting in extravasation of plasma proteins and various blood cells (3, 6) and the development of high interstitial tumor pressures (7). Such characteristics of tumor vasculature can result in hypoxia, concomitant radiation resistance (8), and compromised delivery that limits efficacy of cytotoxic chemotherapeutics (9). Collectively, these conditions present barriers to the delivery of high molecular weight agents such as monoclonal antibodies and their diagnostic or therapeutic conjugates (10, 11).

There are numerous reports demonstrating that external beam radiation can induce alterations in tumor blood vessels (12–15). Radiation has been demonstrated to affect microvasculature permeability and to influence vascular tumor volume (12–14). More salient to our studies are the observations of enhanced efficacy of radiolabeled monoclonal antibodies resulting from irradiation of implanted hepatomas wherein increased differential uptake was noted for tumor versus normal liver (15). Other studies have reported applying a combination of external beam radiation with radiolabeled monoclonal antibodies with varying results (16–20). Results and interpretations have been varied in part due to the considerable variation in the studies, e.g., in vitro versus in vivo, antibody, isotope, disease, fractionated versus single dose external beam radiation, and preclinical versus clinical (15–20). Whereas it seems clear that positive effects are to be gained, numerous questions remain unresolved, and the ability to monitor, measure, and effectively exploit these effects is an attractive goal. Thus, development of appropriate technology to image and monitor changes in tumor vasculature permeability noninvasively are important goals for improving cancer treatment.

With respect to noninvasive approaches to assess tumor leakiness and permeability, a series of gadolinium- (Gd) labeled dendrimers of varying sizes has been synthesized recently (21–23). Dendrimers are a class of highly ordered spherical polymers of which there are a vast array of types, chemical structures, and functional groups. Two types of dendrimers, the polyamidoamine (24) and the dianaminobutane dendrimers (25, 26), are commercially available. These polymeric compounds are highly soluble in aqueous solution and possess a unique surface topology of primary amino groups (24, 27). The defined structure and large number of available surface amino groups of these dendrimers have promoted their use as substrates for the attachment of large numbers of chelating agents to a single antibody molecule (27–31). As noted above, three gadolinium-labeled polyamidoamine dendrimers (generation-4, -6, and -8; G4, G6, G8) ranging in size from 6 to 13 nm have been synthesized recently (21–23). Preliminary studies with these dendrimers indicated that the generation-8 polyamidoamine gadolinium dendrimer (G8-Gd-D; 13 nm), when injected into tumor-bearing animals and imaged using dynamic magnetic resonance imaging, exhibited enhancement associated with tumor vasculature, whereas
the analogous, but smaller G6-Gd-D (10 nm) demonstrated greater evidence of tumor tissue delineation (23). Thus, in this specific model, the cusp between normal tumor vasculature leakage was established to be below the molecular size of the G8-Gd-D validating its use as a tool to explore the impact of agents on tumor permeability. The advantages associated with the use of macromolecular contrast agents versus low molecular weight agents for applications relating to blood vessel imaging have been discussed (32–34). Additionally, the molecular size of the G8-Gd-D is comparable with an IgG molecule thereby bringing relevance to antibody-mediated diagnostics and therapeutics. Herein, changes of tumor vessel permeability imposed by ionizing radiation are assessed by dynamic magnetic resonance imaging using the macromolecular G8-Gd-D. The findings confirm that radiation can alter tumor permeability and that macromolecular Gd-labeled dendrimers coupled with dynamic magnetic resonance imaging provide a novel and effective means to interrogate tumor vessel permeability.

MATERIALS AND METHODS

Preparation of G8-Gd-D Magnetic Resonance Imaging Contrast Agent with Gd(III). Polymidoamine dendrimer (Dendritech, Inc., Midland, MI), G8, ethylenediamine core, 1,024 surface primary amines (MW = 233,383 Da) was used in this study. The dendrimer-IB4M conjugate was prepared and gadolinium complex formation performed as reported previously (23). In brief, G8 dendrimer was conjugated with excess bifunctional chelating agent 2-(p-isothiocyanatobenzyl)-6-methyl-diethyleneaminopentaacetic acid (p-SCN-IB4M; refs. 23, 35). Dendrimer labeling and purity was assessed with a Beckman Coulter high-performance liquid chromatography system equipped with a Tosoh Biosep G3000SW column. Elemental analyses were performed by Galbraith Laboratories Inc. (Knoxville, TN). Multi-angle light scattering measurements were performed by Wyatt Technology Corp. (Santa Barbara, CA). The macromolecular contrast agent was purified as reported previously (23) and freeze-dried thereafter. The amount of chelated Gd(III) atoms in the preparation was determined to be 9.2% w/w by inductivity-coupled plasma atomic emission spectrometer (Galbraith Labs, Inc.). The contrast agent was dissolved to 6 μmol Gd/mL with phosphate buffer (pH 7.2) and was adjusted to physiologic osmolarity with solid NaCl. A 100-μL aliquot of this stock solution was used per mouse.

Animal Preparation. Female C3H mice, produced by the National Cancer Institute Animal Production Area (Frederick, MD), were acquired for this study. The mice were 6 to 8 weeks of age at the time of experimentation and weighed 20 to 30 grams. All of the magnetic resonance imaging experiments were carried out under the aegis of a protocol approved by the National Cancer Institute Animal Care and Use Committee and were in compliance with the Guide for the Care and Use Of Laboratory Animals. Squamous cell carcinoma (SCCVII) cells were injected subcutaneously as a single-cell suspension of 10^6 cells in the right hind leg (36). A preliminary image was performed with a 1.7-cm diameter tumor. All of the imaging experiments thereafter were conducted with tumors of 8-mm mean diameter (~275 mm^3) at ~8 days after injection.

The goal was to have 5 mice in each group for each study. Seven mice were randomly prepared for each group (2 extra), and then 5 selected by tumor size to minimize tumor growth as a variable within each set. Data for those mice that were alive and had also completed the dynamic scan up to the 20-minute time point have been included. Three sets of dynamic data were eliminated because they were incomplete through this time period due to instrumentation difficulties during the dynamic scan.

In preparation for imaging experiments, a tail vein was cannulated with a 30-gauge needle before induction of anesthesia. Anesthesia was induced with 2.0% isoflurane and maintained at 1.5% isoflurane carried by air at 750 mL/min delivered via a nosepiece. An anesthetic delivery/scavenging device was continuously used throughout the experiment. For immobilization within the resonator, the legs of each mouse were secured within a custom-built holder to standardize the position of the tumor-bearing leg relative to the rest of the body. Core body temperature was monitored throughout the imaging experiments by a YSI (Dayton, OH) temperature probe connected to a Digi-Sense Temperature Controller R/S (Cole Parmer, Vernon Hills, IL). The temperature probe was introduced rectally up to a depth of 2 cm and fixed during imaging. The magnetic resonance imaging coil was fully wrapped with a water bath temperature-controlled heating blanket to maintain a core body temperature of 37°C ± 1°C. Immediately after completing the imaging study, all of the mice were sacrificed and dissected to visually inspect the tumor especially for intense necrosis and hemorrhage. The first three tumors were harvested for histology in all of the groups studied.

For radiation studies, mice were placed in customized Plexiglass jigs that allow for immobilization of the right leg without the use of anesthetic. The tumor-bearing right leg was irradiated using an Eldorado 60Co teletherapy unit (Theragnostics International Ltd., Kanata, Ontario, Canada, formerly Atomic Energy of Canada, Ltd.) at dose rates between 200 and 250 cGy/min. Decay corrections were performed monthly, and full electron equilibrium was ensured for all of the irradiations. Special care was taken to avoid irradiation of the body using lead shields specifically designed for these mouse jigs. Tumors were irradiated with single radiation doses and imaged as a function of time postirradiation. Fractionated radiation doses (5 daily doses of 2, 3, or 5 Gy) were administered, and tumors were imaged 2 hours after the final fraction.

Vessel Density Determination. In a separate study, tumor vessel density was determined for 1-cm diameter SCCVII tumors. Tumors from 5 control mice and 5 irradiated mice (15 Gy single radiation dose) were harvested 24 hours later and cryoprotected in 30% sucrose in PBS. The tumors were embedded in OCT medium, snap frozen, and cut in 5-μm sections. Immunohistochemistry was performed using the ABC method (Vector Laboratories, Burlingame, CA). Briefly, sections were fixed in ice-cold acetone for 10 minutes followed by blocking in normal rabbit serum. Samples were incubated overnight at 4°C with rat antimonous PECAM-1 (1:50 dilution, PharMingen, San Diego, CA) followed by sequential incubations with mouse-adsorbed biotinylated rabbit antirat IgG (1:100 dilution, Vector Laboratories) for 30 minutes at room temperature and Vector ABC-AP reagent for 30 minutes at room temperature. Vector Red substrate with Levamisole solution was applied
for 10 minutes followed by dehydration and mounting. Samples were imaged with a DMRXA light microscope (Leica Co., Wetzlar, Germany) and captured with IP Lab software (Scanalytics, Inc., Fairfax, VA). Multiple high-powered fields (×400 magnification) of each sample were analyzed for mean vessel density using NIH Image software.

**Dynamic Magnetic Resonance Imaging.** Dynamic magnetic resonance imaging of the tumor-bearing mice were performed after injection of 0.03 mmol Gd/kg of G8-Gd-D using a 4.7-Tesla, 40 cm horizontal bore Bruker Biospec system (Bruker Biospin, Billerica, MA). All of the dynamic magnetic resonance images were obtained with 1-inch birdcage-shape surface coils. The mice were fixed in the coil in a mouse holder, and multislice two-dimensional gradient-echo images (two-dimensional gefi-tomo sequence) were acquired with a 200-ms repetition time and a 5-ms echo time with a 90° flip angle. Two excitation pulses were used to obtain an optimal signal. Each image data set consisted of eight 1-mm slices with a 0.05-mm gap and displayed in a 256 × 256 matrix over a 5-cm field of view. Each frame of the dynamic images took 102 seconds. Dynamic images were taken every 2 minutes from 0 to 20 minutes (11 frames) initiated immediately after injection of the contrast agents. Images were obtained immediately after injection of contrast agent and were acquired in 111 seconds. The zero time point is defined as completion of the first scan. Regions of interest ranging from 32 to 67 pixels were selected from that part of the tumor with maximum enhancement on a well-centered slice in the tumor. Regions of interest were deliberately set to specifically exclude intratumor vessels or necrotic areas, inclusion of which would clearly compromise observation of changes in tumor vessel permeability. In brief, a 67-pixel region of interest was initially planned. Thus, a region of interest containing 67 pixels excluding surface and penetrating tumor vessels, defined as continuous bright structures on the first enhanced image in one slice or in two adjacent slices, was chosen. In those cases where such structures were unavoidable, the region of interest was adjusted to exclude those structures while also being set as large as possible within those same boundary conditions. In ~8-mm tumors measured from the outside, a 67-pixel region of interest covered ~15% to 20% of the tumor area. Therefore, those areas of minimal enhancement assigned as “necrotic” were reasonably eliminated. The histologic correlation of such nonenhancing areas to hemorrhagic, sclerotic necrosis, or degeneration of tumor was reported (5). The increase in signal intensity (SI) in each tumor was calculated according to the following equation: Enhancement = SI post/SI pre, where SI pre is the signal intensity of the regions of interest just before injection of contrast agent.

**Statistical Analysis.** Statistical analysis was performed using the Student’s t test (Statview, Jandel Scientific, San Rafael, CA).

**RESULTS**

**Selection of Tumor Size**

Prior dynamic magnetic resonance imaging studies evaluating three different sizes of dendrimers (G4-Gd-D, G6-Gd-D, and G8-Gd-D) in SCCVII tumors established that G8-Gd-D contrast enhancement was confined to tumor vessels (23). Preliminary experiments were conducted to determine the pattern of G8-Gd-D enhancement in SCCVII tumors pre- and postirradiation. Shown in Fig. 1 is an example of dynamic magnetic resonance images of a relatively large tumor with 1.7 cm diameter (measured over the skin), which had been irradiated (15 Gy) 12 hours before administration of G8-Gd-D. The images show considerable heterogeneity with respect to G8-Gd-D enhancement over the 20-minute time course. Distinct early enhancement in tumor vessels was observed. There were discrete distinct areas of the tumor where little or no enhancement occurred, and there were areas that exhibited diffuse enhancement. These

**Fig. 1** Dynamic magnetic resonance images with G8-Gd-D of a relatively large SCCVII tumor (16 mm in diameter) 12 hours after γ-irradiation (15 Gy). Images were obtained immediately after injection of contrast agent and were acquired in 111 seconds. The zero time point is defined as completion of the first scan.
preliminary studies directed the use of smaller tumors (~8 mm in diameter) and established a framework for regions of interest determination for subsequent studies. In ~8-mm tumors measured from the outside, a 67-pixel region of interest covered ~15% to 20% of the tumor area. Therefore, those areas of minimal enhancement assigned as necrotic were reasonably eliminated.

**Permeability Change by Ionizing Radiation Using Dynamic Magnetic Resonance Imaging**

**Single Dose.** On this basis, SCCVII tumors (~8 mm in diameter) were exposed to a range of radiation doses (2–15 Gy, single dose), and magnetic resonance imaging studies were conducted with G8-Gd-D after 24 hours. As shown in Fig. 2, tumor enhancement with G8-Gd-D as a function of time after administration demonstrates a radiation dose-dependent increase. *, significant differences (P < 0.01) as compared with control group that received no radiation. Images were obtained immediately after injection of contrast agent and were acquired in 111 seconds. The zero time point is defined as completion of the first scan; bars, ±SD.

G8-Gd-D enhancement for tumors receiving single radiation doses of 2 and 5 Gy was indistinguishable from controls (Fig. 2). There is an increase in enhancement after irradiation with 10 Gy from 1.148 ± 0.061 from the zero time point up to 1.524 ± 0.069 at 14 minutes, which amounts to an 32.8% increase (P = 0.00002). An examination of the data for the increased enhancement ratio over time for the 5, 10, and 15 Gy data reveals slopes of 0.74, 2.5, and 4.4 (10^2 for all), respectively. We interpret this result then to indicate a dose-dependent response. Tumors exposed to 15 Gy exhibited a clear, statistically significant (P < 0.01) enhancement in G8-Gd-D uptake as compared with control tumors. On the basis of this observation, a time course study was conducted using 15 Gy. To quantitatively examine the dependence of enhancement in the tumor on the time after irradiation, enhancement values were obtained in the selected regions of interest at several time points. The results are displayed in Figs. 3 and 4, and representative scans from this study are shown in Fig. 5. The results show that after a 15-Gy treatment, tumor tissue enhancement by G8-Gd-D significantly increased beginning at 7 hours and continued to increase thereafter until 24 hours (P < 0.01). By 36 hours after treatment, G8-Gd-D enhancement of tumor tissue had returned essentially.

![Fig. 2](image-url) Time-intensity curves of G8-Gd-D-enhanced dynamic magnetic resonance imaging. Enhancement ratios 24 hours after γ-irradiation with single doses from 2 to 15 Gy to the tumor-bearing leg are presented. Tumor tissue enhancement by G8-Gd-D as a function of time after administration demonstrates a radiation dose-dependent increase. *, significant differences (P < 0.01) as compared with control group that received no radiation. Images were obtained immediately after injection of contrast agent and were acquired in 111 seconds. The zero time point is defined as completion of the first scan; bars, ±SD.

![Fig. 3](image-url) Time-intensity curves of G8-Gd-D-enhanced dynamic magnetic resonance imaging. The time dependent effects of γ-irradiation (15 Gy) on tumor tissue enhancement by G8-Gd-D are shown. Magnetic resonance images were acquired beginning at 2 hours after irradiation and continued at various intervals until 48 hours when enhancement by G8-Gd-D decreased to below control values. *, significant differences (P < 0.01) as compared with control group that received no radiation. Images were obtained immediately after injection of contrast agent and were acquired in 111 seconds. The zero time point is defined as completion of the first scan; bars, ±SD.
to control values at 48 hours after treatment, was actually below control values (Fig. 3). The time course change of G8-Gd-D enhancement after radiation treatment is summarized in Fig. 4. The slopes of time-intensity curves at 4, 7, 12, 24, and 36 hours after radiation were significantly steeper than that of nonirradiated control group (Fig. 4; P < 0.01). As shown in Fig. 5, at different times after radiation treatment, maximum tumor enhancement by G8-Gd-D is clearly evident at 24 hours after radiation treatment. Because a significant increase in enhancement by G8-Gd-D was observed 24 hours after radiation treatment, alterations in tumor microvessel density might have occurred as a result of the radiation. Representative micrographs of SCCVII tumor sections stained with H&E and for PECAM-1 are presented in Fig. 6. No morphologic changes are apparent in the H&E-stained tumors 24 hours after irradiation (15 Gy; Fig. 6B) as compared with tumor from the nonirradiated controls (Fig. 6A). There are few alterations in tumor cell morphology clustered around the microvessels evident in the micrograph of 48 hours after irradiation (Fig. 6C), and scattered macrophages and eosinophils can be discerned (short arrows). The integrity of blood vessels (long arrows) appears intact and delineated. The endothelial cell morphology, pattern, and contact remains defined. Immunohistochemical staining of the tumor sections for PECAM-1 preirradiation (Fig. 6D) and at 24 hours (Fig. 6E) postirradiation (15 Gy) indicated no significant differences in either the pattern or presence of the endothelial cell marker. Furthermore, no significant differences in the mean vessel density between control and irradiated sections were noted (data not shown).

**Fractionated Doses.** Lastly, a series of experiments were conducted to determine whether tumor tissue enhancement by G8-Gd-D could be obtained or increased by fractionated radiation treatment protocols, analogous to conventional radiotherapy as delivered clinically. Groups of animals were exposed to daily fractions of 2, 3, or 5 Gy for 5 days, and tumor tissue enhancement evaluated 2 hours after the final fraction was delivered (Fig. 7). The results obtained for single doses of 10 and 15 Gy are included in Fig. 7 as a reference. None of the fractionated radiation protocols resulted in enhancement com-

![Fig. 4](https://cancerres.aacrjournals.org) - Slopes of time-intensity curves of G8-Gd-D enhanced dynamic magnetic resonance imaging at the various time points after irradiation (15 Gy). *, significant differences (P < 0.01) as compared with control no radiation group; bars, ±SD.

![Fig. 5](https://cancerres.aacrjournals.org) - Representative pre- and post-enhanced magnetic resonance images. Magnetic resonance images of the SCCVII tumor are shown at selected time points pre- and postirradiation (15 Gy). Corresponding micrographs of the H&E staining are presented for histologic comparison of the effects on the xenografts (magnification ×4). (ROI, regions of interest)
parable with that obtained using single doses of 10 or 15 Gy. In fact, the tumor tissue enhancement for the 5 Gy 5 fractions group was considerably less than that of the controls. Tumor volume over the 5-day treatment period was unchanged for the 2 Gy and 3 Gy protocol as compared with nonirradiated controls. However, tumor growth was substantially inhibited by this fractionated 5 Gy protocol. The initial tumor volume on day 8 in these mice was 364 ± 65 mm$^3$. The average volume of control tumors on day 13 was 1,236 ± 384 mm$^3$ as compared with 533 ± 84 mm$^3$ for tumors in the 5 Gy fractionated dose group.

**DISCUSSION**

The transvascular transport of large molecular weight molecules in tumors is complex (37). Various factors characterize the unorganized nature of tumor blood vessels including lack of functional lymphatics, blunt ends and tortuous bends, fenestrations and abnormal pores leading to tumor vessel “leakiness” (38), and high interstitial pressures (39). These parameters taken in conjunction with the leakiness of tumor vessels provided a basis for a hypothesis that for a given specific tumor type an appropriate size macromolecular dendrimer-based magnetic resonance imaging agent could be created that would be retained within tumor vessels. The appropriateness of application of macromolecular magnetic resonance imaging agents has been well discussed, and their inherent advantages over low molecular weight agents, high relaxivity and sensitivity, retention in vasculature, and relatively slow clearance are established (32–34). This hypothesis was confirmed in part by our prior experience with G6-Gd-D wherein this particular dendrimer was demonstrated to be confined to blood vessels as small as ~200 μm (21). Following in this direction, a recent dynamic magnetic resonance imaging study reported a comparison of G6-Gd-D versus the G8-Gd-D injected into mice bearing a SCCVII tumor. The latter dendrimer-IB4M conjugate, G8-Gd-D, has a molecular weight of 960 kD and is 13 nm in diameter, comparable in physical size to the long diameter of IgG (13 nm). Tumor tissue enhancement was found to continue to increase for the G6-Gd-D over a 20-minute scan indicative of continued leakage from the tumor vasculature into the tumor. However, tumor tissue enhancement was obtained with the G8-Gd-D, which plateaued after a small initial increase that occurred during the first 1 to 3 minutes after injection (23). Therefore, the shape of the dynamic enhancement tumor curve for G8-Gd-D combined with the visual findings of the magnetic resonance images established that G8-Gd-D was confined to the tumor vessels in this specific tumor model system (23).

As alluded to above, the physical characteristics of macromolecular agents are not comparable with low molecular weight agents. The clearance rate of G8-Gd-D is considerably different from that of a standard magnetic resonance imaging contrast agent, for example, Gd-diethylenetriaminepentaacetic acid or “Magnevist.” The blood half-life of the G8-Gd-D contrast agent is ~2 hours, and tissue half-life (wash-out) is ~6 hours (22). Execution of repeated dosing within the 48-hour time frame after study is impossible for the dynamic study, because significant levels of the initial dose of G8-Gd-D remain in the tumor tissue. Thus, whereas it may be possible to do multiple imaging acquisitions with low molecular weight contrast agents via repeated administration, this option is not viable using the G8-Gd-D within the constraints of most animal care facilities.

The spatial resolution of γ-radioimmunoscintigraphy is
Tumor Permeability Altered by Ionizing Radiation

Figure 7: Effect of fractionated doses of radiation on G8-Gd-D enhanced dynamic magnetic resonance imaging. Mice bearing SCCVII tumor xenografts were treated 5 times (daily) with 2, 3, or 5 Gy. Magnetic resonance images were taken 2 hours after the final radiation dose. For comparison, the results of single doses of 10 Gy and 15 Gy are included.

- Significant differences (*P < 0.01) as compared with control no radiation group. Images were obtained immediately after injection of contrast agent and were acquired in 111 seconds. The zero time point is defined as completion of the first scan; bars, ±SD.

The findings of the present study confirm that for SCCVII tumors large single radiation doses transiently increased permeability without an apparent detectable change in microvessel density. This general observation is consistent with prior reports demonstrating enhanced radioimmunotherapy efficacy and monoclonal antibody uptake resulting from external beam irradiation. Whereas the mechanism of this process is unknown, one possible mechanism that has been proposed is radiation-mediated damage to tumor endothelial cells (12). Endothelial damage resulting in clefts or simple gross disruption of cells whereby transvascular trafficking of macromolecules would be permitted has been noted (12). Tumor endothelial cell apoptosis after radiation treatment has also been shown to be an important factor in tumor response to ionizing radiation (56). Transient loss of tumor endothelial cells lining blood vessels could provide openings in the endothelium facilitating entry of macromolecules. Restoration of damaged or missing endothelial cells by circulating stem cells (57) or repair of existing vacancies through platelet aggregation could then explain the transient nature of the macromolecule permeability. Radiation treatment is known to induce adhesion or sticking of leukocytes to the endothelium of normal tissues and to a lesser extent in tumors (58). However, in this study changes in morphology were noted in the tumor cells surrounding the microvessels but not in endothelial cells. Furthermore, this change was not apparent until 48 hours after irradiation. The tumor cells adjacent to these vessels are more likely to be radiosensitive, because oxygen and nutrients would be more readily available to these cells, and they would be proliferating at a higher rate than tumor cells distal from the vessels (8).

Vessel permeability might be transiently affected by the local accumulation of leukocytes. Recruitment of leukocytes and subsequent production of cytokines is typical of an inflammatory response to trauma such as radiation and results in vessel leakage. For example, systemically delivered interleukin 2 or...
tumor necrosis factor α is reported to induce vascular permeability (59). Another factor that might contribute to radiation-induced vessel permeability is the induction of vascular permeability factor/vascular endothelial growth factor. Increased permeability of microvessels to macromolecules by vascular permeability factor/vascular endothelial growth factor (60), coupled with tumor vascular endothelial growth factor expression induction by radiation treatment (61), may contribute to enhanced vessel permeability. Lastly, tumors are known to exhibit high interstitial fluid pressure, which poses a barrier for macromolecule transport into tissue (39). Radiation treatment also decreases tumor interstitial fluid pressure (62), which may well contribute to enhanced tumor permeability of macromolecules. Clearly, despite the substantial literature record of both observations and proposed mechanisms, the need for well-defined studies addressing all of these potential contributing factors remains to be executed to fully understand the molecular mechanisms underlying all of these results. While not the primary objective of this specific study, the goal of creating a noninvasive research tool to study both the effect of radiation on vascular permeability and dynamics of the “biological window” opening and closing in tumor model systems has been reached.

In conclusion, there clearly are numerous potential applications for the imaging strategy and agent described here as research tools in both preclinical and clinical arenas. There are several clinical settings wherein large single radiation doses are delivered to tumors including intraoperative radiation therapy, stereotactic radiosurgery, and high dose rate brachytherapy. Dynamic magnetic resonance imaging coupled with G8-Gd-D before treatment and thereafter could establish and confirm alterations in tumor permeability. On the basis of increased tumor permeability resulting from radiation treatment or for that matter from chemotherapeutics or biologics, the application of suitable radiolabeled antibodies or other anticancer agents could be administered based on optimal timing derived from imaging studies. Studies to exploit these possibilities are currently being designed and will be reported in due course.

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