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**Original Research**

# In Vivo Studies of Gd-DTPA-Monoclonal Antibody and Gd-Porphyrins: Potential Magnetic Resonance Imaging Contrast Agents for Melanoma

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**New tumor-specific contrast agents for clinical imaging and therapy for cancer are required. To this end Gd-H (Gd-hematoporphyrin), Gd-TCP (Gd-tetra-carboranylmethoxyphenyl-porphyrin), Gd-DTPA-WM53, and Gd-DTPA-9.2.27 were synthesized and administered by systemic injection to nude mice with human melanoma (MM-138) xenografts. The biodistribution $T_1$ relaxation times and magnetic resonance (MR) image signal enhancement of the contrast agents are presented for the first time and compared for each group of five mice. A change (20%) in $T_1$ relaxation times of water in human melanoma tumor xenografts was revealed 24 hours after injection of the labeled immunoconjugate Gd-DTPA-9.2.27. The percent of injected antibody or gadolinium that localized to the tumor was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) to be approximately 35%. A higher concentration of gadolinium was achieved compared with nonspecific compounds, indicating selective delivery of Gd-DTPA-9.2.27 to the melanoma xenografts. Porphyrin-based contrast agents (Gd-H and Gd-TCP) also showed significant uptake in melanomas. The uptake of Gd-TCP by the tumor was sufficient to deliver boron atoms into the tumor, making possible dual use for both MR imaging (MRI) and boron neutron capture therapy (BNCT). The linear relationship found between the paramagnetic contribution to the relaxation rates and contrast agent concentration allows quantitative studies of paramagnetic contrast agent uptake. J. Magn. Reson. Imaging 2001;14: 169–174. © 2001 Wiley-Liss, Inc.**

**Index terms:** MRI; monoclonal antibody; Gd-DTPA; Gd-porphyrins; melanoma

MAGNETIC RESONANCE IMAGING (MRI) using varied pulse sequences provides a sharp contrast between tissues with different intrinsic $T_1$ or $T_2$ relaxation times. By exploiting the differences in relaxation times, images have been produced that provide previously unobtainable physiologic and mobility information (1). The use of contrast agents to shorten relaxation times and give enhanced signal intensity (SI) may extend the potential of MRI to the diagnosis of currently subclinical tumors. Two approaches are investigated to increase the specificity of MR image contrast agents by using metalloporphyrins and a monoclonal antibody coupled with Gd-DTPA.

Porphyrins are a unique class of metal chelating agents that have shown selective affinity for a variety of tumors (2). The high water solubility and stability under physiological conditions (3), low propensity for causing photoxicity (4), and intracellular localization in mitochondria for more efficient tumor cell killing (5) are reasons why these complexes have been used as tumor-specific contrast agents. Tumor-specific contrast agents with potential for clinical imaging and therapy for cancer are Gd-hematoporphyrin (gadolinium-[18,13-bis (hydroxyethyl)-3,7,12,17-tetramethyl-21H, 23H porphine-2, 18-dipropionic acid]) and Gd-TCP (gadolinium-tetra-carboranylomethoxyphenyl-porphyrin).

In a second approach, monoclonal antibodies against leukemia and melanoma are used. These are chelated with Gd to form the Gd immunoconjugates Gd-DTPA-WM53 and Gd-DTPA-9.2.27. These agents are tested in the nude mice model with a human melanoma (MM-138) xenograft to investigate their pharmacokinetics. The biodistribution $T_1$ relaxation times and signal enhancement of the contrast agents are presented and compared.

## MATERIALS AND METHODS

### *Monoclonal Antibodies*

Two monoclonal antibodies are used: monoclonal antibody 9.2.27 (specific for melanoma cell line MM-138) and monoclonal antibody WM53 (specific for leukemia cell line HL-60) as the control.

### *Tumor-Specific Contrast Agents*

The preparation method has been reported by Rizvi et al. (6) and is a modification of the method of Hnatowich et al. (7). Cyclic anhydride DTPA (cDTPAa, 0.1 mg) was dissolved in chloroform (1 mL) and was degassed under a stream of nitrogen for 1 hour. 9.2.27 antibody solu-

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tion (2 mg, 1mL) was added and the mixture incubated at 0° C for 45 minutes. The mole ratio of DTPA to 9.2.27 was 20:1. The resulting solution was loaded onto a PD-10 column, Sephadex GM-25 (10 × 1 cm, Pharmacia, Biotech), and eluted with 0.5 M sodium acetate (pH 5.5), collecting 2 mL. The concentration of 9.2.27 in the final solution was determined by protein estimation as 2 mg/mL.

The DTPA-WM53 conjugate was prepared in a manner similar to that of DTPA-9.2.27.

### *Conjugates Labeled With Gd*

The following is the method used for insertion of gadolinium (8) and is the optimized procedure.

Gadolinium(III) chloride hexahydrate (1.8 mg) was dissolved in 1 mL of distilled water. To this solution, DTPA-9.2.27 (4 mg, 2 mL) was added. The pH was adjusted to 5 by the addition of 1 M sodium acetate. After stirring for 1 hour at room temperature, the solution was added to a PD-10 column and eluted with sodium chloride (0.15 M, pH = 5), collecting 1-mL fractions. The fourth and fifth fractions were combined to yield 2 mL of pure Gd-DTPA-9.2.27 solution (protein estimate = 2 mg/mL, [Gd] = 0.47 mM).

The Gd-DTPA-WM53 compound was prepared in manner similar to that of Gd-DTPA-9.2.27 (protein estimate = 3 mg/mL, [Gd] = 0.51 mM).

### *Porphyrin-Based Contrast Agents*

Gd-TCP was synthesized for the first time in this laboratory (9) and (15 mg, 0.010 mmol) was dissolved in 1 mL of cremophor EL (CRM) and 2 mL of 1,2-propanediol (PrG). This solution was transferred into a 10-mL volumetric flask, and a 0.9% saline solution was added to the mark. This gave a final concentration of 1.0 mM.

Gadolinium(III) nitrate hexahydrate (0.30 g, 0.66 mmol) was dissolved in 2 mL of distilled water. Hematoporphyrin powder (0.40 g, 0.66 mmol) was suspended in 2 mL of distilled water and was added to the gadolinium solution and refluxed until the solution became homogeneous. The solution was allowed to cool to room temperature. This solution was reduced to 1 mL under reduced pressure with heating. The resulting white solid was filtered, washed carefully with ice-cold water (2 × 0.5 mL), and dried in the oven at 80° C.

### *Injected Dose*

The animal studies were approved by the UNSW Animal Care and Ethics Committee. Seven groups of five mice were used (age, 6–8 weeks; mean weight, 20 g). Each group was housed five mice per cage in a humidity- and temperature-controlled isolated animal house at St. George Hospital. All mice were fed sterilized standard mouse chow and water ad libitum.

The human melanoma cell line MM-138, originally derived from human malignant melanoma, was grown in tissue culture and injected subcutaneously into both flanks of nude mice ($2.5 \times 10^6$ cells, 120 μL).

Three to four weeks after tumor implantation, when the tumor diameter was 3–5 mm (mean weight of tumors, 200 mg), mice received an intraperitoneal (i.p.) injection of an MRI contrast agent (discrete compounds $GdCl_3$, Gd-DTPA, Gd-H, and Gd-TCP, and conjugated antibody). All contrast agents were diluted in physiological saline to a final concentration as injected in bolus doses (Table 1). Gd-DTPA-9.2.27 was used as the specific agent and Gd-DTPA-WM53 was used as the nonspecific agent. The last group was a control group. The total injected volume was 200 μL. The animals were killed by an overdose of pentobarbital sodium 24 hours post i.p. injection, followed by the removal of critical organs (tumor, kidney, liver, and spleen). These were minced for MRI and inductively coupled plasma atomic emission spectroscopy (ICP-AES) experiments. The gadolinium concentrations in the tumor and various organs were measured by ICP-AES using the acid digestion method (10).

Table 1
Injected Doses of Contrast Agents Into Each Group of Nude Mice

| Group of mice | Contrast agent | Concentration Gd (mM) | Injected dose of Gd (μmol/gbw mouse) |
|---|---|---|---|
| 1 | $GdCl_3$ | 1.0 | 0.01 |
| 2 | Gd-DTPA | 1.0 | 0.01 |
| 3 | Gd-TCP | 1.0 | 0.01 |
| 4 | Gd-H | 1.0 | 0.01 |
| 5 | Gd-DTPA-9.2.27 | 0.5 | 0.005 |
| 6 | Gd-DTPA-WM53 | 0.5 | 0.005 |
| 7 | Control | – | – |

### *In Vivo Proton Relaxation Times ($T_1$) Determination*

The effect of contrast agents on proton relaxation times was measured in tumors and other harvested organs by an inversion recovery (IR) pulse sequence technique using a 7.0 T Varian *UNITY* Plus (Varian Associated Inc., CA) with a vertical Oxford Instruments magnet of bore size 89 mm using a saddle coil (*DOTY* Scientific Instruments).

### *MR Image SI*

The enhancement effect of all contrast agents on MRI signal was investigated. All images were obtained using the $T_1$-weighted imaging method using the IR pulse sequence technique, with $T_I$ = 200 msec, $T_E$ = 15 msec, $T_R$ = 300 msec, 5-mm slice thickness, 3 × 3 cm field of view (FOV), and a matrix size of 256 × 128. MR image SI was measured by averaging the individual SIs of five selected voxels. Variations in coil tuning (performed manually) caused some changes in SI during the experiments.

### *Gadolinium Concentration Measurements*

All tissues were frozen until used for ICP-AES measurements. The gadolinium content was measured by a ICP-AES (Applied Research Laboratory, UK) instrument according to the method of Tamat et al. (10). The 342.249-nm atomic emission line of Gd was chosen for the ICP-AES analysis. The tissue uptake of Gd was calculated as a percentage of the injected contrast agent percentage of injected dose (% I.D.).

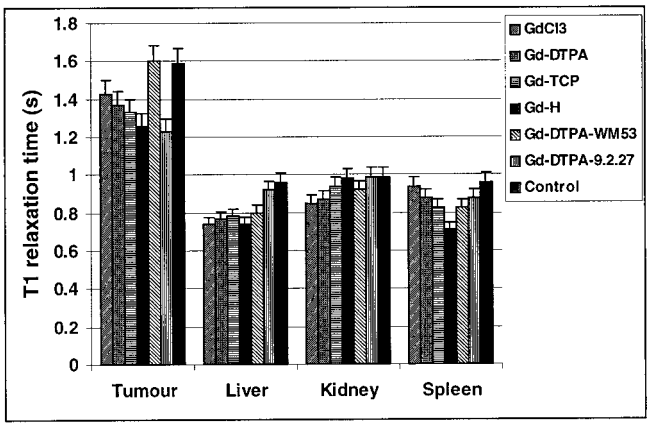


**Figure 1.** In vivo $T_1$ (s) relaxation times of gadolinium compounds in melanoma and in selected organs of nude mice ($N$=5).

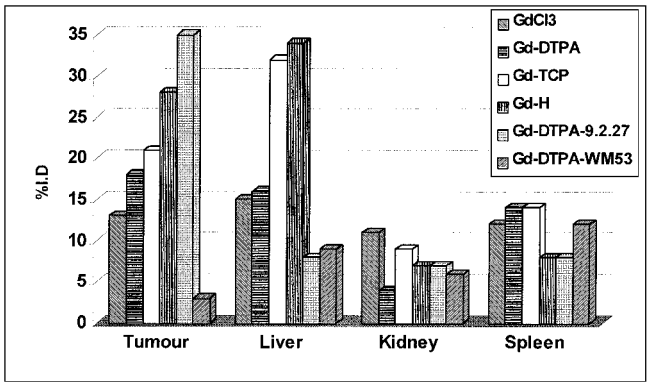


**Figure 3.** Comparison of biodistribution of the gadolinium uptakes in melanoma xenografts in nude mice from harvested organs.

## RESULTS

### *General Aspects*

All mice tolerated the procedures well, including tumor growth and response to contrast agents. No adverse effects were observed after injection of contrast agents and no animal death was recorded during tumor growth or postinjection.

### *$T_1$ Relaxation Times*

The effect of contrast agents on proton relaxation times ($T_1$) was measured in tumors and the other harvested organs. Table 2 shows the $T_1$ values for organs using different contrast agents and untreated mice (control). The plot of $T_1$ values for removed tissues is shown in Figure 1.

These results reflect the gadolinium concentrations in the tissues reported above. The high uptake of Gd-DTPA-9.2.27 by the tumor resulted in approximately 20% change in the $T_1$ relaxation time of the water in human melanoma xenograft when compared to the $T_1$ value for the control. The nonspecific antibody conjugate, Gd-DTPA-WM53, recorded a $T_1$ value similar to the control. Gd-TCP and Gd-H showed a 16% and 21% decrease in the $T_1$ value for the tumor, respectively relative to the control.

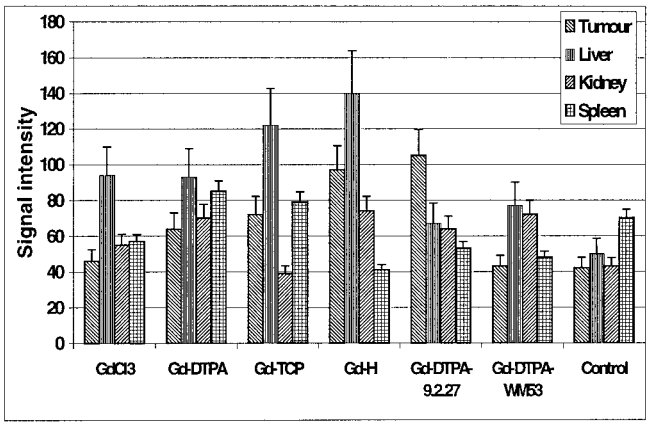


**Figure 2.** MR image signal intensity of tissues 24 hours after injection of different gadolinium compounds ($N$=5).

### *MR Image SI*

MR image SIs for tumor and removed organs and different contrast agents are shown in Figure 2. In the control, the MR image SI for tumors was lower than that recorded for the normal tissues studied. This may result from the $T_1$ relaxation time for the tumor being longer than the other normal tissue, which decreases SI and is consistent with $T_1$ values measured in this work (Table 3).

### *Gadolinium Content of Tissues*

Figure 3 clearly shows that the highest concentration of gadolinium in tumor (35%) was achieved using Gd-DTPA-9.2.27, the antibody conjugate using the specific antibody. This is consistent with the results of the in vitro experiments.

In contrast, the lowest uptake (3%) was found for Gd-DTPA-WM53, the conjugate made using the nonspecific antibody. The antibody has retained its nonspecificity for the melanoma cell upon the attachment of the chelate and gadolinium ions. Tumor uptakes of 21% and 28% of injected gadolinium were recorded for Gd-TCP and Gd-H, respectively.

## DISCUSSION

Quantitative determination of paramagnetic contrast agents in tissues will help to obtain optimal MR image intensity (11). Most commonly, the information is obtained from relative MR image intensity changes. This method suffers from poor accuracy because the relaxivity of the agents in the tissues is generally unknown. The more direct and accurate method involves killing and dissecting the animals following administration of contrast agents. The concentration is then determined by ICP-AES. In this study we report two in vivo methods to determine the relative concentration of contrast agents in tissues following administration, using paramagnetic chelates and monoclonal antibodies.

Table 2
Average $T_1$ Relaxation Times of Different Organs in Groups of 5 of Nude Mice Xenografted With Human Melanoma*

| Contrast agent | $T_1$ relaxation times (s)[a] | | | |
|---|---|---|---|---|
| | Tumour | Liver | Kidney | Spleen |
| $GdCl_3$ | 1.43 ± 0.03 | 0.74 ± 0.02 | 0.85 ± 0.01 | 0.84 ± 0.03 |
| Gd-DTPA | 1.37 ± 0.02 | 0.77 ± 0.01 | 0.87 ± 0.02 | 0.88 ± 0.02 |
| Gd-TCP | 1.33 ± 0.02 | 0.78 ± 0.02 | 0.94 ± 0.02 | 0.83 ± 0.02 |
| Gd-H | 1.26 ± 0.01 | 0.74 ± 0.02 | 0.98 ± 0.03 | 0.71 ± 0.03 |
| Gd-DTPA-WM53 | 1.60 ± 0.03 | 0.80 ± 0.02 | 0.92 ± 0.01 | 0.83 ± 0.02 |
| Gd-DTPA-9.2.27 | 1.23 ± 0.04 | 0.92 ± 0.03 | 0.99 ± 0.01 | 0.88 ± 0.02 |
| Control | 1.59 ± 0.03 | 0.96 ± 0.01 | 0.99 ± 0.04 | 0.96 ± 0.02 |

*Tissues were removed 24-hr after injection of different contrast agents.
[a]Data are mean ± SEM of values obtained from five mice.

Paramagnetic chelates using the endogenous porphyrin ring as the chelating agent are a promising and interesting family group of potential MRI contrast agents (12). Gd-porphyrins were synthesized using TCP and hematoporphyrin and investigated as MRI contrast agents.

It is also possible to achieve shortening of $T_1$ in the tumor xenograft by means of gadolinium-labeled tumor-specific antibody. Essential to this experiment was the development of a conjugate gadolinium complex. After forming the protein-DTPA complexes, approximately four atoms of Gd were bound per molecule of 9.2.27 antibody. This conjugate was used as a melanoma-specific detection MRI contrast agent.

### *$T_1$ Relaxation Times*

The results showed that the $T_1$ relaxation time of the tumor was significantly greater than that for normal tissues. The general theory that $T_1$ values are longer in tumors was confirmed by this animal study. This difference is reported to arise from an increase in water content and the large extracellular volumes of the cancerous tissues that elevate the $T_1$ values (13). From the point of view of comparison between $T_1$ values of tumors in excised tumor and live tumor tissues, results indicate that the $T_1$ relaxation time is lower in excised tumor tissue than in live animal tissue and may decrease with time after excision due to further loss of water by evaporation (14,15).

The decreases in the $T_1$ values of the other discrete gadolinium complexes were in line with the concentrations of gadolinium absorbed by the tumors. These reductions in $T_1$ values of the tumor upon addition of contrast agent are highly significant, even though the concentration of agents used in this study (0.005–0.01 mmol/kg) is much lower than the doses of Gd-DTPA commonly used as a contrast agent in clinical MRI (0.1 mmol/kg).

As can be seen from Figure 1, all contrast agents show shortening of the $T_1$ relaxation time in the liver, except for Gd-DTPA-9.2.27. The most significant decrease in $T_1$ relaxation times of the liver occurred when treated with porphyrin compounds. These results are consistent with the gadolinium concentrations found in these organs.

Figure 1 also illustrates that the reduction of $T_1$ relaxation times in the kidney using conjugated antibody agents is less than that of discrete contrast agents. The most effective modification of $T_1$ was observed in the kidney after administration of $GdCl_3$. This is probably due to free gadolinium and its urinary excretion. The smallest $T_1$ value in the spleen was obtained for Gd-hematoporphyrin. In contrast, the largest $T_1$ relaxation time was observed for $GdCl_3$. No significant change in the $T_1$ value was observed for the other contrast agents.

### *MRI SI*

The enhancement of the MR image SI after injection of tumor-specific contrast agents causes changes in the $T_1$ values. The highest SI was observed for the tumor upon injection of Gd-DTPA-9.2.27, reflecting the shortening of $T_1$ relaxation times and the maximum accumulation of the contrast agent in the tumor. The porphyrin-based contrast agents, Gd-H and Gd-TCP, showed good enhancement of the signal of the tumor. The enhancement effect of the porphyrin complexes in this study is in good agreement with that reported previously by conjugation of Gd-DTPA with porphyrins under in vivo conditions in mice (16). The enhancement effect of the image intensity of the tumor following administration of Gd-DTPA showed the potential application of this contrast agent. Although the uptake by the tumor of $GdCl_3$ was significant, the concentration of gadolinium in the tumor was too low for signal enhancement with this contrast agent.

These results also illustrate the MRI contrast-enhancing capabilities of Gd-TCP. This signal enhancement of Gd-TCP is indicative, but not proof of, the delivery of boron atoms into the cancer cells. Then Gd-TCP could be a dual-use compound, as it can enhance contrast between tumor and normal tissues in MR images and be potentially effective as an agent for boron neutron capture therapy (BNCT).

The liver showed the greatest enhancement for both porphyrin agents and the accumulation of gadolinium complex in this organ. The high MR image SI was recorded in the liver for Gd-DTPA and $GdCl_3$, consistent with both the $T_1$ relaxation time and the uptake dose determination. The enhancement recorded in the liver using these porphyrin compounds was greater than that for monoclonal conjugated agents. This indicates the accumulation of the nonimmunoconjugated gadolinium complexes into the liver rather than the tumor.

Table 3
Signal Intensity of Different Organs 24 hr after Injection of Different Contrast Agents

| Contrast agent | Signal Intensity* | | | |
|---|---|---|---|---|
| | Tumor | Liver | Kidney | Spleen |
| $GdCl_3$ | 48 3 | 93 5 | 57 3 | 58 3 |
| Gd-DTPA | 63 4 | 92 4 | 70 5 | 83 5 |
| Gd-TCP | 71 3 | 121 6 | 40 2 | 80 4 |
| Gd-H | 98 5 | 140 9 | 65 4 | 40 2 |
| Gd-DTPA-9.2.27 | 105 7 | 66 3 | 63 3 | 55 3 |
| Gd-DTPA-WM53 | 42 2 | 77 5 | 73 5 | 46 2 |
| Control | 42 3 | 50 3 | 42 2 | 70 4 |

*Data are mean SEM of values obtained from five measurements.

The MRI SI in the kidney is lower than that for other organs (Fig. 2). This is again due to the properties of renal excretion of gadolinium-based contrast agent as hydrophilic compounds. The graphs of MR image SI and harvested organs show the enhancement effect for two discrete compounds, Gd-DTPA and Gd-TCP. No significant intensification was observed in the spleen for the other contrast agents.

### *Gadolinium Content of Tissue*

The gadolinium concentration in tumor and other tissues was measured by ICP-AES, and the results compared with the percent of injected Gd localizing in the tumor (Fig. 3). The percent of injected specific antibody or gadolinium (35%) that localized into the tumor was higher than that reported for other antibodies in a mouse tumor (1,17). This shows that our antibody conjugate has a high potential for use as a contrast agent for the detection of melanoma.

A higher concentration of Gd was achieved in tumor than with nonspecific compounds ($GdCl_3$, Gd-DTPA-WM53), indicating selective delivery of Gd-DTPA-9.2.27 to the melanoma xenografts. Significant Gd accumulated in the liver, spleen, and kidney, probably reflecting clearance and metabolism of the Gd complexes. Figure 3 also indicated that the uptake of Gd-DTPA in the tumor was approximately half of that for conjugated specific antibody, Gd-DTPA-9.2.27. This shows the benefit of conjugation of Gd-DTPA with a monoclonal antibody for specific-tumor detection agents. Interestingly, the uptake by the tumor of $GdCl_3$ was significant.

The porphyrin-based compounds also showed their potential as tumor-specific detection agents. The amount of boron introduced into the tumor by Gd-TCP was found to be 24 μg. This compares well with other values for related complexes (18), is sufficient for BNCT, and proves the potential application of the Gd-TCP in the dual roles of MRI agent and boron delivery agent for BNCT.

Generally, a significant gadolinium complex was retained in the liver except for the antibody conjugates, Gd-DTPA-9.2.27 and Gd-DTPA-WM53. The liver retained the highest amount of gadolinium for the Gd-porphyrins. At 24 hours postinjection, the gadolinium content of Gd-porphyrins was at least two times greater in the liver than that of Gd immunoconjugates. Some of the gadolinium found in the liver might represent gadolinium dissociated from the DTPA. It is known that free gadolinium accumulates in the liver and this may explain some of the high uptake in the reticuloendothelial organ (19). This is in agreement with results of gadolinium content observations reported by other researchers (4,20). Significant gadolinium accumulation in the liver, spleen, and kidney probably reflected clearance and metabolism of the gadolinium complex (21).

As these results indicate, the smallest amount of gadolinium was observed in the kidney at 24 hours postinjection. This is due to the properties of gadolinium-based contrast agents, which are hydrophilic, accumulate in the extracellular water of the tissues, and have rapid renal excretion. In this study, 30%–50% of the injected dose disappeared in 24 hours, which is consistent with rapid clearance (21). The uptake of gadolinium in the spleen and kidney showed no significant differences for any of the gadolinium compounds.

The amount of monoclonal antibody injected per kilogram was much greater than the quantities routinely required in nuclear medicine animal experiments (22). Given the low percentage of monoclonal antibody localizing to the tumor and the hazards of injecting increasing amounts of foreign protein, it is doubtful that much higher concentrations of gadolinium in the tumor could be obtained by increasing the amount of antibody injected. Of course, the radioisotopes used in nuclear medicine limits the application of higher doses of contrast agents.

The difference between these data and previous animal experiments (1,11,13) may arise in differences in the pulse sequences, dose of Gd-DTPA-mab used, the type of antibody, and possibly the method of measuring tissue concentration of Gd.

At what time and to what extent the MRI can be obtained depends on the accessibility of contrast agents to the tumors. Other researchers (23,24) found that the enhancement effect of Gd-DTPA-mab on MRI continued for 24 or 72 hours after injection. Our results show a good accumulation of the 9.2.27 conjugate in tumor at 24 hours postinjection. This means that Gd-DTPA-9.2.27 is suitable as a diagnostic melanoma contrast agent for MRI. Improvement of MRI techniques and novel contrast agents can also help MRI as a powerful diagnostic modality for malignant and benign tissues.

## CONCLUSIONS

Tissue-specific agents are required for improved MRI of melanomas in regional lymph nodes. We have shown that MRI with Gd-DTPA-9.2.27 offers the advantage of tissue contrast enhancement and precise anatomic localization of the tumors, as a substantial enhancement effect was observed in nude mice with melanoma xenografts. Further developments in MRI contrast agents, in combination with improved imaging techniques, may lead to novel applications in the diagnostic MRI. In this study, the pharmacokinetics of a paramagnetic contrast agent based on monoclonal antibody 9.2.27 have been studied in a melanoma tumor xenograft model. Further investigations will include conjugating higher numbers of Gd atoms into the chelating agent, using different monoclonal antibodies, and identifying uptake time of Gd in tissues.

Gd-TCP also can be used as a dual probe for MRI contrast agent and as a radiation sensitizer for NCT. This may facilitate tumor detection and treatment planning and allow radiologists and radiation oncologists to better diagnose and define the radiation treatment field. The ability to easily visualize the contrast agents in the tumor may also make it possible to optimize timing of radiation therapy treatments in order to maximize tumor cell kill. These attributes potentially will improve the safety and efficacy of radiation therapy. Both the MRI findings and the metal content measurements in this study are consistent with the previous biodistribution data that the liver is a major organ responsible for the active uptake and prolonged elimination of porphyrin (4,20).

Overall, with the satisfactory low levels of Gd in the liver, kidney, and spleen, and good tumor uptake, Gd-DTPA-mab has considerable promise for further diagnostic applications in MRI. The outcome of this study may help the design of tumor-specific contrast and chemotherapeutic agents. The permeability of macromolecule tracers, such as Gd-DTPA-mab, may provide tissue uptake information for both contrast and chemotherapeutic agents. With current debate on tumor-specific and therapeutic agents for tumor diagnosis and therapy, the permeability of macromolecules in tumor tissues is an essential question that needs to be answered.

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