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Original Article

Novel MR imaging contrast agents for cancer detection

Daryoush Shahbazi-Gahrouei[a]

Abstract

BACKGROUND: Novel potential MR imaging contrast agents Gd-tetra-carboranylmethoxyphenyl-porphyrin (Gd-TCP), Gd-hematoporphyrin (Gd-H), Gd-DTPA-9.2.27 against melanoma, Gd-DTPA-WM53 against leukemia and Gd-DTPA-C595 against breast cancer cells were synthesized and applied to mice with different human cancer cells (melanoma MM-138, leukemia HL-60, breast MCF-7). The relaxivity, the biodistribution, T_1 relaxation times, and signal enhancement of the contrast agents are presented and the results are compared.

METHODS: After preparation of contrast agents, the animal studies were performed. The cells (2×10^6 cells) were injected subcutaneously in the both flanks of mice. Two to three weeks after tumor implantation, when the tumor diameter was 2-4 mm, mice were injected with the different contrast agents. The animals were sacrificed at 24 hr post IP injection followed by removal of critical organs. The T_1 relaxation times and signal intensities of samples were measured using 11.4 T magnetic field and Gd concentration were measured using UV-spectrophotometer.

RESULTS: For Gd-H, the percent of Gd localized to the tumors measured by UV-spect was 28, 23 and 21 in leukemia, melanoma and breast cells, respectively. For Gd-TCP this amount was 21%, 18% and 15%, respectively. For Gd-DTPA-9.2.27, Gd-DTPA-WM53 and Gd-DTPA-C595 approximately 35%, 32% and 27% of gadolinium localized to their specific tumor, respectively.

CONCLUSIONS: The specific studied conjugates showed good tumor uptake in the relevant cell lines and low levels of Gd in the liver, kidney and spleen. The studied agents have considerable promise for further diagnosis applications of MR imaging.

KEYWORDS: Magnetic Resonance, Imaging, Monoclonal Antibody, Contrast Agents, Gadolinium, Early Detection of Cancer.

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Despite its relatively short history, MR imaging has become a major diagnostic tool in all clinical specialities.[1-6] The technique of MR imaging has major advantages over other methods: it is completely non-invasive, does not expose the patient to ionizing radiation, appears to have no side-effects, enables soft tissue imaging and based on current evidence appears to be harmless.[7] In addition, the multi-planar capabilities, high spatial resolution and excellent soft tissue contrast have all contributed to the development of MR imaging as a powerful tool for guiding tumor specific detection procedures.

Relaxation times are the most important parameters for selection of the optimum MR imaging technique.[1-4] Differences between relaxation times of normal and cancer tissues are responsible for the superb soft tissue contrast in this method. Although MR imaging was initially hoped to provide a means of making definitive non-invasive diagnosis, researchers have found that adding contrast agents in many cases improves sensitivity and specificity.[1-7] Lauterbur et al[2] were the first to demonstrate the feasibility of using paramagnetic contrast agents to improve tissue discrimination in MRI. Today, research efforts are concentrated on the delivery of specific T_1 value agents into the tumors.

The discrete contrast agents (Gd-DTPA, $GdCl_3$, Gd-TCP, and Gd-H) and the mono-

[a] Department of Medical Physics and Medical Engineering, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: shahbazi@med.mui.ac.ir

clonal antibody conjugates (Gd-DTPA-9.2.27, Gd-DTPA-C595 and Gd-DTPA-WM53) were tested for their in-vivo uptake. The tumor and selected organs (liver, kidney and spleen) were removed 24-hr after injection of the contrast agents. Gadolinium concentration, T_1 relaxation times, and MR image signal intensity of these organs were determined and the results were compared.

Methods

The study was performed in the Department of Medical Physics, Isfahan University of Medical Sciences (Isfahan, Iran) during 2006-2007. Two new gadolinium complexes of porphyrins were synthesized.[7,8] Briefly, the synthetic TCP (tetra-carboranylmethoxyphenyl-porphyrin) was produced by modifying the method of Miura et al[5] and was inserted with gadolinium to yield Gd-TCP. The naturally occurring porphyrin, hematoporphyrin, was also inserted with gadolinium to yield the neutral species gadolinium-hematoporphyrin (Gd-H).

Monoclonal antibodies, 9.2.27 against melanoma, WM53 against leukemia and C595 against breast cancer cells were conjugated with Gd-DTPA. In summary, the monoclonal antibodies were treated with the cyclic anhydride of DTPA, followed by gadolinium chloride to yield gadolinium antibody DTPA conjugates with concentrations of gadolinium of about 5 mM.[8-10] Solutions of different contrast agents were prepared and the animal studies were performed with mice of 6-8 weeks old with a mean weight of 25 g. The animal studies were approved by the animal care of the School of Medicine. Animals were randomly divided into sixteen groups of five (5 groups for each cell line (5×3) or 15 groups for different 5 contrast agents, and one group as a control). Each group was housed per cage in humidity and temperature controlled, isolated animal house at the School of Medicine, Isfahan University of Medical Sciences.

The human cancer cells (melanoma MM-138, leukemia HL-60, breast MCF-7), (2×10^6 cells, 120 μl) were injected subcutaneously in the both flanks of mice. Two to three weeks after tumor implantation, when the tumor diameter was 2-3 mm, mice were injected with different contrast agents intraperitoneally (i.p). All contrast agents were diluted in physiological saline to a final concentration as injected in bolus doses (0.01 mmol/kg of body weight). The injected volume for all contrast agents were the same (250 μl). The animals were sacrificed by an over-dose of pentobarbital sodium at 24 hr post i.p. injection, followed by removal of tumor, kidney, liver and spleen. The maximum Gd content for tumor uptake was occurred at 24 hr post i.p. injection and this time was selected as sacrificed time.[10]

All samples were prepared using an acid digestion procedure according to the method of Tamat et al.[11] Briefly, to a weighed sample of tissue (60-120 mg) in a polyethylene vial, 0.3 ml of 72% perchloric acid was carefully added and the contents swirled to mix. 0.6 ml of 32% hydrogen peroxide was added and the vials placed in the shaking bath for 5 hr at 23 °C. At this stage, the vial contents were clear and colourless. The samples were diluted to 3 ml of distilled water, and filtered through a 0.45 □m Millipore filter before being introduced into both NMR and UV-spect experiments.[7-10]

The T_1 relaxation times and signal intensities in solution of tumors and other harvested organs were measured using an inversion recovery (IR) sequence (180° rf-τ_i -90° rf-collect FID) technique using 11.4 T (500 MHz, *Bruker instrument, Germany*) located in Tarbiat Modarres University, Tehran, Iran. The repetition time chosen was 2.5 times the estimated T_1. Ten inversion delays (T_1) were used with each increasing by a factor of two. The minimum inversion time used was approximately one-tenth of the estimated T_1 value. The values of echo time and repetition time were also optimized for different samples. Variations in coil tuning (performed manually) caused some changes in signal intensity during the experiments. The gadolinium content of washing

Table 1. Relaxivity values of different MR imaging contrast agents at room temperature (23° C) at magnetic field strength of 11.4 T.

Contrast agents	r_1 ($mM^{-1} s^{-1}$)	r_2 ($mM^{-1} s^{-1}$)
$GdCl_3$	11.8 ± 0.7	14.5 ± 1.2
Gd-DTPA	3.8 ± 0.1	4.6 ± 0.4
Gd-H	16.8 ± 1.2	19.5 ± 1.3
Gd-TCP	33.1 ± 0.3	39.2 ± 0.6
Gd-DTPA-9.2.27	24.1 ± 0.6	30.2 ± 0.2
Gd-DTPA-WM53	23.3 ± 1.4	28.7 ± 1.5
Gd-DTPA-C595	25.7 ± 0.3	32.2 ± 0.7

Solution was measured us-ing UV- spectrophotometer (*Spectronic Gene Sys2, Spectronic Instrument*).

Results

The increment of the water proton relaxation rate per unit concentration of the paramagnetic contrast agent is called the relaxivity (R_1) and is calculated according to the following formula:

$$1/Ti = 1/Ti\ (control) + Ri\ C$$

Where i = {1,2}, Ti is relaxation time of sample, Ti (control) is relaxation time of blank or the system before adding contrast agent, C is concentration of paramagnetic contrast agent or Gd, and Ri is the relaxivity ($mM^{-1}s^{-1}$). The measurement was performed in aqueous solution of different contrast agents (table 1). As can be seen, relaxivity values of the synthetic porphyrin agents (Gd-H and Gd-TCP) are higher than that of clinically used agent (Gd-DTPA).

The decrease in T_1 values of tumor solutions for specific Gd antibody conjugate was larger than that for porphyrin-based agents. Both Gd-TCP and Gd-H showed decreases in the T_1 value for the tumor, respectively (Table 2).

As can be seen in table 3, for Gd-DTPA-9.2.27, Gd-DTPA-WM53 and Gd-DTPA-C595, most of the gadolinium were localized to the tumors and these values was greater than those of Gd content of other organs. For Gd-TCP and Gd-H, the injected gadolinium taken up by the tumor was slightly lower than that for specific antibody conjugates. No retention of gadolinium in the tumor was observed when the non-specific agent was used. Gd-DTPA and $GdCl_3$ showed some uptake significantly less than those of Gd-antibody conjugates and Gd-porphyrin complexes.

Signal enhancement of 110% over the control was observed for Gd-antibody conjugates. Both Gd-H and Gd-TCP also enhanced the signal intensity by 90% and 60%, respectively.

Table 2. T_1 relaxation times of different contrast agents in washing solution after acid digestion method (mean ± SEM of values obtained from an average of five samples).

Compound / Sample	MM-138	HL-90	MCF-7
$GdCl_3$	1.21 ± 0.01	1.19 ± 0.01	1.14 ± 0.03
Gd-DTPA	1.01 ± 0.04	1.10 ± 0.02	1.03 ± 0.02
Gd-TCP	0.81 ± 0.02	0.84 ± 0.02	0.78 ± 0.01
Gd-H	0.76 ± 0.01	0.78 ± 0.03	0.74 ± 0.03
Gd-DTPA-9.2.27	0.72 ± 0.01	-	-
Gd-DTPA-WM53	-	0.72 ± 0.01	-
Gd-DTPA-C595	-	-	0.78 ± 0.05
Control	1.29 ± 0.03	1.12 ± 0.02	1.20 ± 0.01

Table 3. Gadolinium concentration of different contrast agents in washing solution (mean ± SEM of values obtained from an average of five samples).

Compound / Sample	MM-138	HL-90	MCF-7
$GdCl_3$	6.7 ± 0.2	3.0 ± 0.3	5.0 ± 0.1
Gd-DTPA	12.0 ± 0.1	13.0 ± 0.2	11.0 ± 0.2
Gd-H	27.7 ± 0.2	23.0 ± 0.2	21.3 ± 0.2
Gd-TCP	21.0 ± 0.3	18.0 ± 0.4	15.0 ± 0.3
Gd-DTPA-9.2.27	34.8 ± 1.3	-	-
Gd-DTPA-C595	-	-	27.0 ± 0.5
Gd-DTPA-WM53	-	32.0 ± 0.6	-

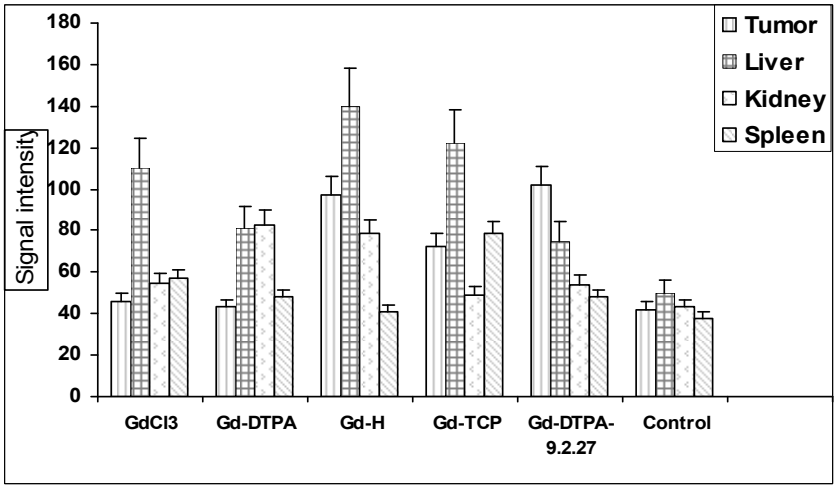

Figure 1. MR image signal intensity of washing solution of melanoma and other organs 24 hr after injection of different gadolinium compounds (n = 5).

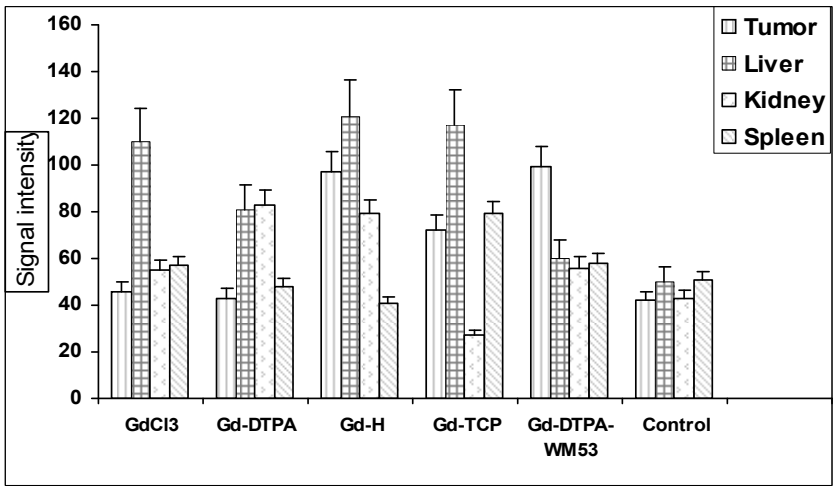

Figure 2. MR image signal intensity of washing solution of leukemia and other organs 24 hr after injection of different gadolinium compounds (n = 5).

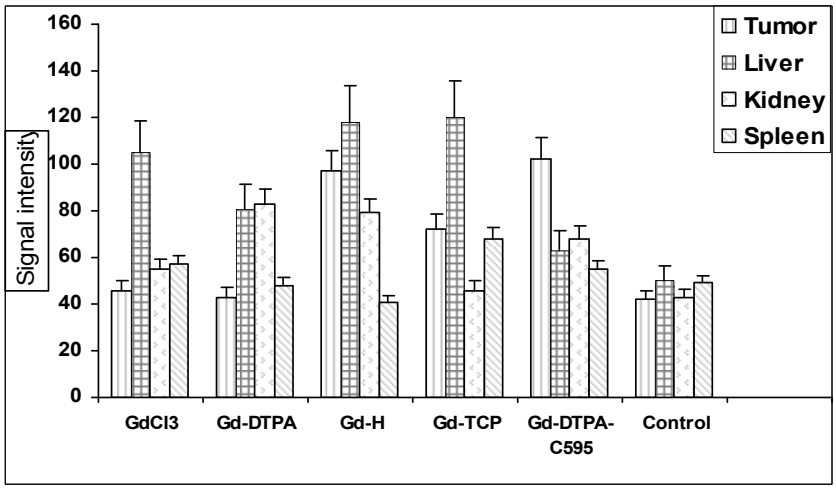

Figure 3. MR image signal intensity of washing solution of breast cells and other organs 24 hr after injection of different gadolinium compounds (n = 5).

The high concentration of Gd in the solution of tumors is indicative of a selective retention of the Gd-antibody conjugates and also indicates that they are promising MR imaging contrast agents for melanoma, leukemia and breast cancer cells detection (Figures 1-3).

Discussion

In aqueous solutions of a variety of gadolinium complex ions, a linear relationship between the paramagnetic ion concentration and relaxation rate was observed, which was consistent with previous works.[7,8] This relationship has been experimentally verified for aqueous solutions of discrete compounds, Gd-DTPA, $GdCl_3$, Gd-H and Gd-TCP. The porphyrin complexes have high relaxivity due to their ability to coordinate several water molecules in aqueous solution. Their high water solubility and stability of Gd based porphyrins under physiological conditions, low propensity for causing phototoxicity, and intracellular localization in mitochondria for more efficient tumor cell killing are reasons why these complexes have been used as new tumor-specific agents in MR imaging.

The relaxation rates of gadolinium conjugated agents were higher than the relaxation rates of Gd-DTPA. Curtet et al[12] observed a similar effect for a Gd-DTPA antibody conjugate. This signifies that Gd-DTPA conjugated to monoclonal antibodies enhances MR image signal intensity at much lower doses (0.005-0.01 mmol/kg Gd) than those currently used (0.01-0.1 mmol/kg) with contrast agents such as Gd-DTPA. The use of antibodies has the double advantage of specificity and efficiency that enables their use at concentrations well below the toxicity threshold, suggesting that they have a major role as contrast agents in MR imaging.

In addition, the influence of the magnetic field strength on both T_1 and T_2 relaxation times was investigated at high magnetic field. A significant increase in T_1 values and a decrease in T_2 values, at magnetic field strengths of 11.4 Tesla were observed when compared to the analogous values at lower magnetic field strength reported by author and colleagues previously.[7,8] Relaxation time measurements in this study were proved the theory of field dependence of relaxation times.[13]

In-vivo studies with human cell lines (MM-138, MCF-7 and HL-60) in mice showed that Gd-DTPA-9.2.27, Gd-DTPA-C595 and Gd-DTPA-WM53 have a high affinity to their specific cancer cells. The result here demonstrated selective tumor uptake for the specific antibody conjugates as compared to the unrelated antibody, whereas their distributions in normal tissues were similar.[14]

Compared to Gd-DTPA, all monoclonal antibody conjugated with Gd showed low uptake

by the liver except the porphyrin complexes whose characteristic is to be very highly uptaken by liver. The biodistribution of all contrast agents studied was similar in the kidney and spleen.

The difference between this data and previous animal experiments[7-10] may arise from 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. By selecting an antibody with a higher affinity for a greater number of antigenic sites per cancer cell, the amount of antibody injected into each mouse can be increased.

The enhancement of the MR image signal intensity after injection of tumor-specific contrast agents was reflected in the changes of T_1 values. The MR image signal intensity of approximately 110% over the control was observed for the tumor upon injection of specific Gd-conjugates monoclonal antibodies, reflecting the shortening of the T_1 relaxation times. After Gd-conjugated agents, the porphyrin-based contrast agents, Gd-H and Gd-TCP, showed good MR image signal enhancement of 100% and 60% in the tumor compared with the control. The liver was the only organ that showed the greatest MR image signal enhancement for both porphyrin-based agents, indicating the accumulation of gadolinium complex in this organ.

Conclusions

MR imaging can be used for diagnosis of cancers followed by administration gadolinium compounds. This approach has been shown to be feasible in animal and cell line studies, and will be applied as appropriate to develop optimal MR imaging in cancer in early stages.

In this study a relationship between Gd contribution to the relaxation times and contrast agent concentration was observed, indicating quantitative studies of agents uptake.

More investigations may include conjugating higher number of gadolinium atoms into the chelating agent, using different monoclonal antibodies, and determination of time point uptake of gadolinium in tissues.

Monoclonal antibodies labeled with gadolinium have been considered in order to effectively target contrast agents to a tumor site. Methods of using MR imaging and monoclonal antibodies conjugated to Gd-DTPA can offer the advantage of tissue contrast enhancement and precise anatomic localization of the tumors.

The findings of this study may help diagnosis of tumors in the early stages. The permeability of monoclonal antibodies conjugated with Gd-DTPA may also provide more information about applying of contrast agents in MR imaging modalities.

Conflict of Interest

Authors have no conflict of interests.

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