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In vitro studies of gadolinium-DTPA conjugated with monoclonal antibodies as cancer-specific magnetic resonance imaging contrast agents

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Abstract

The monoclonal antibodies, 9.2.27 against human melanoma cell lines and WM53 against leukemia cell lines, were conjugated with cyclic anhydride gadolinium-diethylenetriaminepenta-acetic acid (Gd-cDTPAa) and used as tumor-specific contrast agents in magnetic resonance imaging (MRI). The data indicate that Gd-DTPA-9.2.27 in solution decreased the T_1 relaxation of water protons at 7.0 Tesla (300 MHz) in direct proportion to the gadolinium concentration, and this effect was greater than in Gd-DTPA solutions. These conjugates show high specificity for melanoma and leukemia cell lines. T_1 relaxation time at 7.0 Tesla, measured for the melanoma cell line (MM-138) and leukemia cell line (HL-60) after incubation at 37 °C for 4 hr, were significantly decreased (approximately 25%) relative to controls. The gadolinium concentration in cells and washing solutions was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES). A linear relationship was observed between T_1 relaxation rates and gadolinium concentrations obtained by ICP-AES. The ICP-AES results showed no gadolinium uptake in the non-targeted HT-29 colorectal cancer cells.

Key words magnetic resonance imaging, gadolinium, monoclonal antibody, and cancer.

Introduction

The development of contrast agents with tissue-specific enhancement deserves considerable attention because of their potential in earlier and improved diagnosis and in possible therapy. One approach to increasing the specificity of MR image contrast is to use a monoclonal antibody (mab) coupled with contrast agent Gd-DTPA (1-4). The use of antibodies for diagnostic imaging in the search for tumors was established in 1953 by Pressman and Korngold (5), who imaged a tumor in a rat using ^{131}I-labelled polyclonal antibodies. The development of monoclonal antibodies (6) offers the possibility of defined product for routine diagnosis. Mach *et al* (7) published the first description of clinical studies with monoclonal anti-CEA antibodies in 1981.

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MR imaging has three major advantages compared to scintigraphy regarding the application of monoclonal antibodies (mab) as a tumor-specific contrast agent. First, MR imaging avoids the handling of radioisotopes by the use of naturally occurring paramagnetic metals. Second, in MR imaging, the tissue can be imaged before and after administration of the imaging contrast agent. The third advantage of MR imaging is its higher spatial resolution, which permits better localization and differentiation of the tumor (4).

Gadolinium is the element of choice for MR image enhancement due to its high number of unpaired electron (8). The first in-vivo studies with the gadolinium-labeled monoclonal antibody were performed by Unger *et al* (1) and by Andersen-Berg *et al* (2). Gadolinium was attached to the monoclonal antibody via the coupling of anhydride derivatives of DTPA. These studies showed that 100-1,000 gadolinium ions are needed per antibody conjugate are required to achieve concentrations in the tumor sufficient for signal enhancement in MR imaging (8). Curtet *et al* (3) coupled mab to gadolinium using cyclic anhydride method and demonstrated that T_1 relaxation of water protons decreased significantly (by 15%) with mab 19-9, which recognizes human colon adenocarcinoma. They used a 25-Gd-DTPA-antibody conjugate to reduce the effect of immunologic reactivity. Implanted human colon carcinoma

tumors in mice have been successfully imaged by using monoclonal antibodies with a large number of Gd-DTPA molecules attached (9). In recent years, in vivo studies of Gd-DTPA-mab and Gd-porphyrins as MR imaging contrast agents for melanoma detection was investigated in nude mice by author and colleges (10).

Recently, research efforts concentrated on maximizing the delivery of specific T_1 agents to tumors. Gohr-Rosental *et al* (4) reported successful studies of Gd-DTPA monoclonal antibody in subcutaneous tumor in nude mice. One year later, Matsumura *et al* (11) observed MR imaging contrast enhancement by Gd-DTPA-monoclonal antibody in 9L glioma rats. However, the number of Gd attached to the DTPA-protein complex, the effect of chelation on antibody specificity, and the Gd-DTPA-antibody stability is problematic.

The aims of this study are to investigate the conjugation of gadolinium with monoclonal antibody and to observe the contrast enhancement effect of Gd-DTPA-mab as an MR imaging contrast agent. To this end, all samples were tested by inductively coupled plasma atomic emission spectroscopy (ICP-AES) to determine the gadolinium concentration. The effect of the Gd-DTPA-mab on the in vitro conditions and its relaxivity was also investigated.

Materials and methods

Mab 9.2.27 for melanoma

The monoclonal antibody 9.2.27 (specific for melanoma cell line MM-138) was supplied through the Royal Newcastle Hospital. This antibody has a high specificity for all human melanoma cell surfaces and frozen sections of fresh surgical melanoma specimens (12, 13) because of the strong antigenic expression of a 250-kilodalton N-linked glycoprotein and a high molecular weight proteoglycan component larger than 400-kilodaltons.

Mab WM-53 for leukemia

The murine monoclonal antibody WM53, specific for leukemia cell line HL-60, was supplied at Westmead Hospital.

DTPA-mab conjugation

Both conjugates (DTPA-9.2.27 and DTPA-WM53) were prepared in a similar manner. The mab was covalently bound to the DTPA chelating agent (14). Cyclic anhydride DTPA (cDTPAa, 0.1 mg) was dissolved in chloroform (1 ml) and was degassed under a stream of nitrogen for 1 hr. 9.2.27 (2 mg, 1ml) and WM53 (3 mg, 1ml) antibodies solution was added and the mixture incubated at 0 °C for 45 minute. Mole ratio of DTPA to 9.2.27 was 20:1. The resulting solution was loaded on to 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 and WM53 in the final solution was determined by protein estimation as 2 mg/ml and 3 mg/ml, respectively.

Gd-DTPA-mab

The following is an adopted method used for insertion of gadolinium (3,11) 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) and DTPA-WM53 (6 mg, 2 ml) was added separately. The pH was adjusted to 5 by addition of 1-M sodium acetate. After stirring for 1 hr 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 gadolinium conjugates. The gadolinium concentration was (protein estimate = 2 mg/ml, [Gd] = 0.47 mM) in conjugate of Gd-DTPA-9.2.27 solution and (protein estimate = 3 mg/ml, [Gd] = 0.51 mM) in conjugate of Gd-DTPA-WM53 solution.

Protein estimation and Gd attachment to conjugate

All column fractions from the chromatography column (purification) were collected and an aliquot of each fraction was analyzed for concentration of protein and gadolinium. Concentration of protein measured by spectrophotometer UV absorption at 280 nm (SPECTRAmax 250, USA) using standard protein estimation method. An aliquot was examined by both ICP-AES and MR imaging to determine the final amount of coupled protein-gadolinium and gadolinium concentration in the conjugates. The fourth and fifth fractions from the column were combined as a Gd-DTPA-mab tumor specific agent for further studies.

Gadolinium-hematoporphyrin

Gadolinium(III) nitrate hexahydrate (0.30 g, 0.66 mmol) was dissolved in 2 ml of distilled water. Hematoporphyrin ([8,13-bis(1-hydroxyethyl)-3,7,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid]) 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 become 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 ºC. The yield was 0.11 g (21%).

T_1 relaxation times and relaxivity measurements

T_1 relaxation times of samples was measured using 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 the saddle coil (*DOTY* Scientific Instruments). The values of T_I=200 ms, T_E=30 ms and T_R=1000 ms was used for relaxation times measurements. 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:

$$1/T_i = 1/T_{i\,(control)} + R_i\,C$$

where i = {1,2}, T_i is relaxation time of sample, $T_{i\,(control)}$ is relaxation time of blank or the system before addition of

contrast agent, C is concentration of paramagnetic contrast agent or Gd, and R_i is the relaxivity (mM^{-1} s^{-1}). The measurement was performed in aqueous solution of Gd-DTPA-mab.

Conjugation of Gd-DTPA-mab with cells

The specificity of the monoclonal antibody conjugates was tested with the melanoma and leukemia cell lines. The following procedure was performed using solutions of gadolinium in discrete compounds ($GdCl_3$, Gd-DTPA, and Gd-H) and conjugates antibody (Gd-DTPA-WM53 and Gd-DTPA-9.2.27).

The melanoma cell line (MM-138, 2.5×10^6 cells/ml, St. George Hospital) and leukemia cell line (HL-60, 2.5×10^6 cells/ml, St. George Hospital) were incubated with Gd-DTPA-9.2.27 and Gd-DTPA-WM53 for 4 hr at 37 °C, respectively. Colorectal cell line (HT-29) was used as a non-specific control. The incubation time for all contrast agents was chosen as 4 hr, sufficient time for saturated attachment of agents to the cell membranes. After incubation, all the cells were washed twice with PBS/2%FCS, followed by centrifugation, then resuspended in PBS/2%FCS solutions. All samples and solutions were tested by both ICP-AES and NMR.

Determination of gadolinium concentration by ICP-AES

The gadolinium content was measured based on an acid digestion procedure using ICP-AES (Applied Research Laboratory, UK) instrument according to the method of Tamat *et al* (15). Briefly, to a weighed sample of tissue (50-100 mg) in a polyethylene vial, 0.3 ml of 72% perchloric acid was carefully added and the contents swirled to mix. Then 0.6 ml of 32% hydrogen peroxide was added and the vials placed in the shaking bath for 5 hr at 25 °C. At this stage, the vial contents were cleared and colorless. The samples were diluted with 3 ml of distilled water and filtered through a 0.45 μm Millipore filter (Altech Association Inc., Australia) before being introduced into the ICP-AES. The 342.249 nm atomic emission line of gadolinium was chosen for the ICP-AES analysis. A separate solution of $GdCl_3$ (0.1 mg/ml) was prepared containing perchloric acid and hydrogen peroxide, but without the heating and filtering steps.

Results

Effect of Gd-DTPA-9.2.27 on relaxation time T_1

Figure 1 and Table 1 show the effect of Gd-DTPA-mab on water relaxation time T_1 at room temperature. In these experiments, the concentration of DTPA-9.2.27 was kept constant (0.01 mM), and the amount of gadolinium concentration bound to DTPA-9.2.27 was varied. As this figure shows increases in the number of bound gadolinium ions resulted in a decrease in T_1 relaxation time. At high concentrations of gadolinium, the effect on T_1 relaxation time was reduced (Fig. 1).

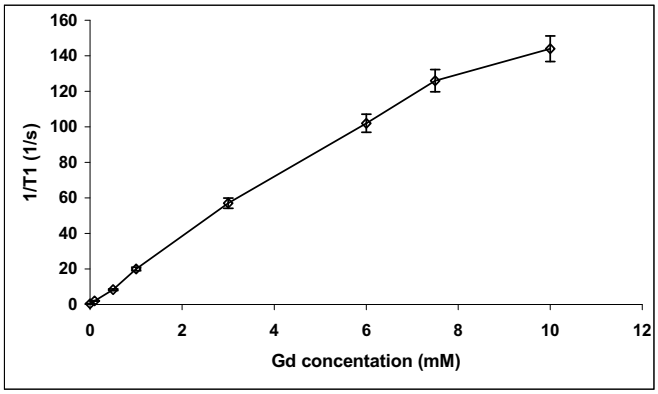

Figure 1. *Effect of gadolinium insertion into DTPA-9.2.27 on T_1 relaxation times in aqueous solution.*

DTPA-9.2.27 (mM)	$GdCl_3$ (mM)	T_1 (ms)	$1/T_1$ (1/s)
0.01	0.0	3003 ± 45	0.33 ± 0.05
0.01	0.1	500 ± 12	2 ± 0.05
0.01	0.5	121 ± 8.2	8.3 ± 0.1
0.01	1.0	50 ± 6	20 ± 0.4
0.01	3.0	17.5 ± 1.4	57 ± 0.5
0.01	6.0	9.8 ± 0.5	102 ± 0.5
0.01	7.5	7.9 ± 0.6	126 ± 0.9
0.01	10.0	6.8 ± 0.4	147 ± 0.8

Table 1. *$1/T_1$ relaxation rates of water for different number of gadolinium bound to Gd-DTPA-9.2.27 in aqueous solution. Gadolinium ions inserted by reaction of DTPA-9.2.27 with $GdCl_3$.*

Relaxivity of Gd-DTPA-9.2.27

The T_1 relaxation rate of the solution of Gd-DTPA-9.2.27 was measured to be 13.5 s^{-1} (T_1 = 0.074 s) at a concentration of gadolinium of 0.47 mM. The T_1 relaxation rate of a solution of Gd-DTPA of the same concentration was 0.55 s^{-1}. This relaxivity was higher than that of Gd-DTPA alone (8). However, Gd-DTPA-9.2.27 was more effective than Gd-DTPA (3.7 mM^{-1} s^{-1}) in reducing relaxation times.

Determination of gadolinium uptake by Melanoma cells (MM-138)

T_1 relaxation time and gadolinium concentration measurements performed using discrete contrast agents $GdCl_3$, Gd-DTPA, Gd-H, and conjugates antibody Gd-DTPA-9.2.27 specific for melanoma cell line with MM-138 (2.5×10^6 cells/ml). Colorectal cell line, HT-29, (2.5×10^6 cells/ml) was used as non-specific target cells for 9.2.27 antibody. Unlabelled conjugates were used as negative or control. To determine the amount of gadolinium which the cells would not bind, samples of washing solutions were collected and analyzed by both MR imaging and ICP-AES. The amount of gadolinium in digested cell solutions and

Contrast agent	Wt. of Gd (mg) in cells*	Wt. of Gd (mg) in washing solution*	T_1 (s) in cells	Initial [Gd] (mM)
$GdCl_3$	0.000 ± 0.002	0.144 ± 0.012	2.06 ± 0.01	1
Control	0.000 ± 0.002	0.000 ± 0.002	1.80 ± 0.02	0
Gd-DTPA	0.052 ± 0.003	0.097 ± 0.006	1.55 ± 0.01	1
Gd-H	0.082 ± 0.004	0.064 ± 0.005	1.40 ± 0.04	1
Gd-DTPA-9.2.27[a]	0.071 ± 0.004	0.012 ± 0.003	1.32 ± 0.03	0.5

Table 2. *The amount of gadolinium contrast agents and T_1 relaxation time in melanoma cell line (MM-138) and washing solution. a. Solution obtained from chromatography column. *. Data obtained from five measurement.*

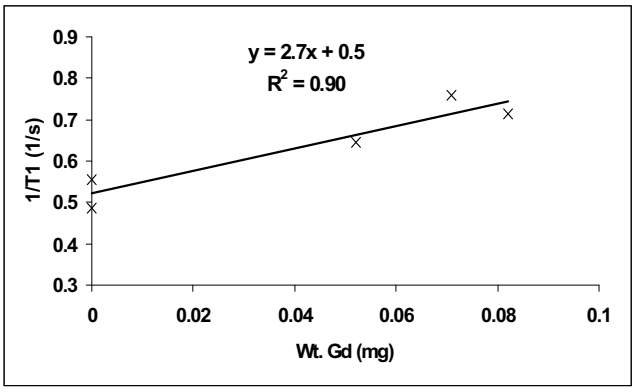

Figure 2. *Relationship between T_1 relaxation rates and gadolinium concentrations in melanoma (MM-138), using MR imaging and ICP-AES measurement respectively.*

washing solutions was measured by ICP-AES and MR imaging is shown in Table 2.

For $GdCl_3$, 100% of this material was found in the washing solution. This indicated that, during incubation, $GdCl_3$ remained in solution and did not become fixed to the melanoma cells. For the monoclonal antibody conjugate, Gd-DTPA-9.2.27, 85% of this material was taken up by (or become attached to) the cell during incubation. Gd-DTPA showed some uptake into the melanoma cell membranes with incubation, however, the amount was significantly lower than that of the monoclonal antibody conjugate. Gd-H showed a greater uptake into the melanoma cells compared to Gd-DTPA, but still not as high as the uptake of the antibody conjugate.

The T_1 relaxation times of the intact cells reflected the amount of gadolinium absorbed into or fixed to the cell membranes. As Table 2 shows the highest amount of gadolinium and the lowest T_1 relaxation time is observed for the specific conjugated monoclonal antibody, Gd-DTPA-9.2.27. Figure 2 shows the graph of T_1 relaxation rates plotted against the gadolinium quantity.

Table 3 shows the percentage of gadolinium distributed between cells and washing solutions, as measured by ICP-AES. The specific conjugate added to melanoma cells compared to controls shows a significant variation of 25% in the T_1 relaxation time.

Sample	Cell lines	%[Gd] in cells*	%[Gd] in washing solutions*	T_1 (s) of cells
Gd-DTPA-9.2.27[a]	MM-138	85 ± 14	15 ± 5	1.32 ± 0.03
Gd-DTPA-9.2.27[a]	HT-29	0.0 ± 2	100 ± 16	2.50 ± 0.06
$GdCl_3$	MM-138	0.0 ± 1	100 ± 14	2.06 ± 0.01
Gd-DTPA	MM-138	35 ± 3	65 ± 7	1.55 ± 0.02
Gd-H	MM-138	56 ± 4	44 ± 6	1.40 ± 0.04
Control	MM-138	0.0 ± 1	-	1.80 ± 0.02
Control	HT-29	0.0 ± 2	-	2.45 ± 0.06
PBS/2%FCS	-	0.0 ± 1	-	3.65 ± 0.05

Table3. *Distribution of gadolinium between cells and washing solutions for MM-138 and HT-29 cells when treated with different contrast agents. a. Solution obtained from chromatography column. *. Data obtained from five measurement.*

Leukemia cell uptake of contrast agents

Several studies have assumed that the decrease of T_1 relaxation time in melanoma is attributed to the presence of melanin (17-19). To test this assumption and to ascertain whether decreases in T_1 values may have a more general usefulness as tumor-specific agent, the specificity of the monoclonal antibody conjugate Gd-DTPA-WM53 was investigated with leukemia cell line (HL-60). Colorectal cell line (HT-29) was also used as a non-specific for this conjugate. The procedure and conditions was similar to the previous section. The results of T_1 relaxation times and the amount of gadolinium in cells and in washing solution are shown in Table 4. The effect of shortening relaxation time were observed in leukemia cell for both specific Gd-DTPA-WM53 (25%) and Gd-H (21%). It can be seen that the decreases in T_1 values when using Gd-DTPA-mab compared with Gd-DTPA are approximately 12% for leukemia and 15% for melanoma. In order to compare the mab-conjugate and other contrast agents, incubation of leukemia cells in the discrete contrast agents $GdCl_3$, Gd-DTPA, and Gd-H was also tested. HL-60 with no incubation with contrast agents was used as control.

The T_1 relaxation times of the cells and the amount of gadolinium attached to the leukemia cell are shown in Table 4. The relationship between relaxation rates ($1/T_1$) and gadolinium content of cells is presented in Figure 3. This graph is consistent with the observations made for the T_1 values of melanoma cells treated contrast agents. A relationship exists but the difference in relaxivity of the complexes does not allow for a higher degree of linearity in the graph.

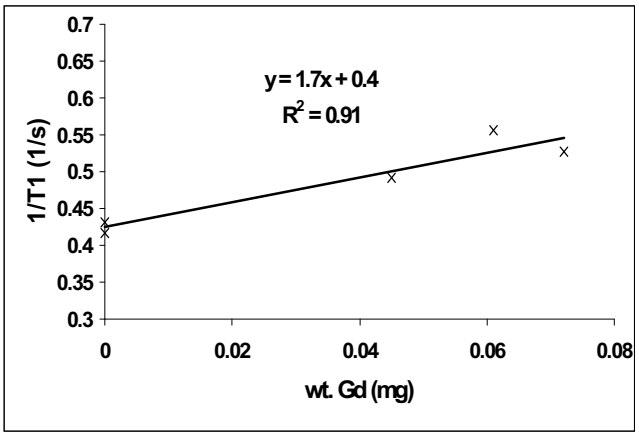

Figure 3. *Relationship between T1 relaxation rates and gadolinium concentrations in leukemia (HL-60), using MR imaging and ICP-AES measurement respectively.*

Results in Table 5 for gadolinium content are presented as a percentage of the initial gadolinium quantity added to the leukemia cells before incubation. The highest amount of gadolinium (78%) into the cells was achieved by monoclonal antibody conjugate Gd-DTPA-WM53, and as expected the smallest amount of gadolinium concentration (22%) in washing solutions observed, approving that the most of gadolinium complex was found into the cells. Again for the non-specific target cell, HT-29, and HL-60 as negative control, there was no gadolinium in cells and most of gadolinium was observed in the washing solutions.

Contrast agent	Wt. of Gd (mg) in cells*	Wt. of Gd (mg) in washing solution*	T_1 (s) in cells	Initial [Gd] (mM)
$GdCl_3$	0.000 ± 0.002	0.154 ± 0.010	2.32 ± 0.01	1
Control	0.000 ± 0.002	0.000 ± 0.002	2.40 ± 0.01	0
Gd-DTPA	0.045 ± 0.003	0.100 ± 0.014	2.03 ± 0.03	1
Gd-H	0.072 ± 0.003	0.078 ± 0.009	1.90 ± 0.04	1
Gd-DTPA-WM53[a]	0.061 ± 0.003	0.017 ± 0.003	1.80 ± 0.04	0.5

Table 4. *The amount of gadolinium material in leukemia cell line (HL-60) and washing solution. a: Solution obtained from chromatography column. *. Data obtained from five measurement.*

Sample	Cell lines	%[Gd] in cells	%[Gd] in washing solution	T_1 (ms) of cells
Gd-DTPA-WM53[a]	HL-60	78 ± 4	22 ± 3	1.80 ± 0.04
Gd-DTPA-WM53[a]	HT-29	0 ± 2	100 ± 14	2.50 ± 0.06
$GdCl_3$	HL-60	0 ± 2	100 ± 10	2.32 ± 0.01
Gd-DTPA	HL-60	31 ± 2	69 ± 6	2.03 ± 0.03
Gd-H	HL-60	48 ± 3	52 ± 4	1.90 ± 0.04
Control	HL-60	0 ± 2	-	2.40 ± 0.02
Control	HT-29	0 ± 1	-	2.45 ± 0.06
PBS/2%FCS	-	0 ± 1	-	3.65 ± 0.05

Table 5. *Distribution of gadolinium between cells and washing solutions for HL-60 and HT-29 cells when treated with different contrast agents. a. Solution obtained from chromatography column.*

Discussion

Several approaches might be considered to increase gadolinium concentration in the tumor. It is possible to increase the amount of DTPA coupling per molecule of mab, although with a corresponding loss of immunoreactivity (1,3,10). In order to improve the quality of imaging and increase efficacy of therapy using radiometal-labeled mab, cyclic DTPA anhydride has been developed and well characterised. Hnatowich *et al* (14) reported a successful conjugation of DTPA to human albumin (18.8 mg/ml, 2.8×10^{-4} M) using cDTPAa as an acylating agent. In this work this agent was used to conjugate DTPA to a practical concentration (300 μg/ml) of monoclonal melanoma antibody (9.2.27). The choice of anhydride is sensible. Cyclic DTPA anhydride forms one of the strongest chelates known for a large number of metals (14).

ICP-AES measurements

Although several methods are available in measuring trace metals of tissue, the ICP-AES method was found to highly accurate and sensitive for the analysis of gadolinium (19).

MR imaging of samples of Gd-DTPA in HL-60 cells demonstrated that concentration of gadolinium necessary for significant enhancement on the 7.0 Tesla magnetic field strength was 0.01 mM (Table 5). This concentration of gadolinium is approximately the same as that required for gadolinium in the form of Gd-DTPA unattached to protein and roughly corresponds to that reported by other researchers as giving significant enhancement on MR imaging (10,20).

T_1 relaxation time measurements

Before assessing the potential clinical value of gadolinium coupled monoclonal antibody for MR imaging of tumors, it was necessary to ensure that the antibody did not modify the paramagnetic properties of gadolinium in the complex. The studies performed on aqueous solutions of Gd-DTPA confirmed that T_1 is inversely proportional to the concentration of the paramagnetic agent. When gadolinium was conjugated with the antibody by means of the chelator cDTPAa, a linear relation was observed between the reversal of T_1 relaxation time and the gadolinium concentration.

The effect of covalently conjugating one (or more) gadolinium ions to a slowly tumbling macromolecule such as an antibody or synthetic protein is to increase the correlation time τ_c and enhance relaxivity. Monoclonal antibodies labeled with gadolinium have been considered in order to effectively target cancer cells with the contrast agent to.

The effect of gadolinium on the relaxation time was greater when it was coupled with antibody. In fact, gadolinium binding on this macromolecule caused an increase in the correlation time (τ_c) of the paramagnetic complex, thus leading to an increase in the speed of relaxation (20, 21). This effect arises when a paramagnetic ion or complex is bound to a protein or macromolecular because of an increased τ_r, and hence τ_c. The rotational correlation time τ_r increases as a function of molecular volume and leads to increased relaxivity.

The uptake of Gd-DTPA-WM53 in leukemia cells was measured to test the assumption that decreases in T_1 values in melanoma cells is due to the presence of melanin. The results for leukemia show similar reduction of T_1 values to those for melanoma cells so cannot be ascribed to melanin. Of course, differences may also arise because antibodies have different affinity to conjugation with DTPA and different specificity for target cells.

Melanoma cell uptake of contrast agent

The results of incubation of melanoma cells with discrete compounds and conjugates monoclonal antibody contrast agents showed that no was gadolinium uptake using $GdCl_3$. This also showed that any gadolinium released from their complexes either in the discrete compounds or the antibody conjugate will end up in the washing solutions. Therefore, any gadolinium found inside the cell lines must have been delivered to the cell membrane as the intact gadolinium complex. The assumption was made that the volume of the melanoma cells has not changed during their incubation with the contrast agents. Hence, the amount of gadolinium reflects the concentration of gadolinium in the treated cells. The lack of any uptake by HT-29 of this material was consistent with this finding the antibody remaining selective to melanoma. These results are in good agreement with in vivo findings in nude mice (10).

The relationship between relaxation rates and gadolinium concentration is well-established in homogeneous solutions and is used extensively in this work (21). However, the extension of this relationship for cells and tissue has great potential. If the relationship between relaxation rates and gadolinium concentration is valid in tissues and biological media, then the concentration of contrast agent could be determined in situ. This work establishes that a relationship exists between the T_1 relaxation rates of melanoma cells and gadolinium concentrations.

Figure 2 showed the existence of a relationship between relaxation rates and gadolinium concentration, but it is clearly not high linear. This non-linearly is due to the differences in relaxivity between the complexes. The higher than expected T_1 value for melanoma cells treated with Gd-DTPA-9.2.27 arise from its higher relaxation rate compared to Gd-DTP. This result is also consistent with the complexes remaining intact inside the melanoma cell membrane after incubation. More information is required to enable gadolinium concentration in tumor cells to be determined in this manner.

Leukemia cell uptake of contrast agent

The results of incubation of leukemia cells with discrete compounds and conjugates monoclonal antibody contrast agents showed the specificity of Gd-DTPA-WM53

tumor-specific contrast agent and its potential as a MR imaging probe for the detection of leukemia.

For Gd-DTPA some gadolinium (31%) attachment was observed in the cells, but most (69%) was found in washing solutions. This result was consistent with the observation for melanoma for which 65% of gadolinium was found in washing solution. The results in Table 5 also showed that for Gd-H approximately half of the amount of gadolinium was found into the leukemia cell.

Both measurements in washing solutions and cells using ICP-AES and T_1 relaxation times confirmed the high specificity of the specific conjugates for targeted cells (Gd-DTPA-9.2.27 for melanoma and Gd-DTPA-WM53 for leukemia) compared to controls. The results also demonstrated that monoclonal antibody conjugates with Gd-DTPA have the potential to deliver these agents to the target tumor. No gadolinium was detected for non-specific colorectal (HT-29) cells for both antibody conjugates and all gadolinium was found in washing solutions. Differences between relaxation rates of melanoma and leukemia cells arise from differences in the type of cells and antibodies.

Conclusions

Specific targeting of MR imaging contrast agents demands a detailed knowledge of the properties of the agent used. For $GdCl_3$, gadolinium was not detected in the cancer cells and all gadolinium was found in washing solutions. For Gd-DTPA and Gd-H, some gadolinium was attached to the cells. However, a larger fraction of gadolinium complex was observed in washing solutions.

A 25% reduction T_1 relaxation time was observed in both melanoma and leukemia cell lines for specific conjugated mab compared to controls. This was confirmed by observation of gadolinium by ICP-AES in the acid digested cells. While the relationship between contrast agent concentration and relaxation rates of both melanoma and leukemia cell lines is established, its extension into tumours and biological media is not as clear. Nevertheless, this technique has potential for the non-invasive in vivo determination of gadolinium concentration.

As results showed, the smallest T_1 relaxation time, reflecting the highest gadolinium accumulation was observed for both specific conjugated monoclonal antibodies (Gd-DTPA-9.2.27 for melanoma and Gd-DTPA-WM53 for leukemia). This is because the monoclonal antibody binds the gadolinium to the cell membrane and/or internalizes the gadolinium into the cell membranes. As the distribution volume increases, the tissue concentration of contrast agent increases, which can result in reducing their relaxation times.

These results indicates that Gd-DTPA-9.2.27 and Gd-DTPA-WM53 may have affinity to their specific cancer cells, indicating that these monoclonal antibody conjugates are potential MR imaging contrast agents for detection of cancer at early stages. Gd-H also has a similar potential application as a tumor-specific MR imaging contrast agent.

The findings of this study can be summarized as follows: Firstly, The optimal measures of the conjugation of DTPA anhydride and monoclonal antibody were as follows: pH = 5.5, cyclic DTPA anhydride/antibody = 20:1. In the conventional labeling method, the ratio of attached chelates to monoclonal antibody is usually >1 in order to provide enough chelating groups for a good labeling yield. Secondly, The data indicate that Gd-DTPA-9.2.27 in solution decreased the T_1 relaxation of water protons at 7.0 Tesla (300 MHz) in direct proportion to the gadolinium concentration, and this effect was greater than in Gd-DTPA solutions.

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