Gadolinium-cDTPAa conjugated with melanoma monoclonal antibody 9.2.27 as a melanoma specific MRI contrast agent

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Introduction

The development of tissue-specific contrast agents deserves considerable attention because of their specificity to the tumor. In recent years, research efforts are concentrated on maximizing the delivery of specific T_1 agents to tumor (1). One approach to increasing the specificity of MR image contrast is to use a monoclonal antibody (McAb) coupled with Gd-DTPA. 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 1) to determine the optimal concentrations for the conjugation of gadolinium and 9.2.27 antibody; 2) to observe the contrast enhancement effect of tissue-specificity with MRI contrast agent-McAb. To this end, the effects of the Gd-DTPA-antibody on the in vitro relaxivity are reported. All samples were tested by inductively coupled plasma atomic emission spectroscopy (ICP-AES) to determine the Gd concentration.

Materials and Methods

The McAb 9.2.27 against human melanoma cell lines was conjugated with cyclic anhydride diethylenetriaminepentaacetic acid (cDTPAa) chelating agent as described by Hnatowich et al (2). $GdCl_3$ (37 mg/mL, Sigma, Aldrich), 10-fold excess of DTPA-McAb, was dissolved in distilled water and added to the cDTPAa-McAb conjugate. The pH was adjusted to 5 by addition of 1 M sodium acetate. After stirring for 1 hour at room temperature, the solution was purified through a sephadex GM-25 column (10×1cm) and eluted with sodium chloride (0.15 M, pH = 5).

The melanoma cell line (MM-138, 2.5×10^6 cells/mL) was incubated with Gd-DTPA-9.2.27 for 4 hr at 37 $^{\circ}$C. Colorectal cell line (HT-29) was used as a non-specific. 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.

The relation between relaxation rates versus [Gd] was obtained using solutions of $GdCl_3$. T_1 values were obtained on a 7.0 T (Varian *UNITY* Plus) using the saddle coil with a vertical Oxford Instruments magnet of bore size 89 mm. The T_1 was measured using an inversion recovery (IR) pulse sequence with 32 incremental values. The gadolinium content was measured based on an acid digestion procedure using ICP-AES (Applied Research Laboratory, UK) according to the method of Tamat *et al* (3). The 342.249 nm atomic emission line of Gd was chosen for the ICP-AES analysis.

Results

The data indicate that Gd-cDTPAa-9.2.27 in solution decreased the T_1 relaxation of water protons at 7.0 T in direct proportion to the gadolinium concentration, and this effect was greater than in Gd-DTPA and $GdCl_3$ solutions. The standard curve shows a very high correlation between inverse T_1 relaxation times and Gd concentration [Gd]. The ICP-AES results showed no Gd in the control HT-29 cells. The relaxivity of Gd-DTPA-9.2.27 were found to be 12.7 $mM^{-1}s^{-1}$. Results of T_1 values for different contrast agents in MM-138 and HT-29 are given in table 1. T_1 values decreased (approximately 25%) relative to control.

Table 1. T_1 values of different contrast agents in MM-138 and HT-29 cell lines.

Sample	Cell lines	Initial [Gd] mM	T_1 (msec)
Gd-DTPA-9.2.27	MM-138	50	1250±20
Gd-DTPA-9.2.27	HT-29	50	2500±34
$GdCl_3$	MM-138	5	2060±10
Gd-DTPA	MM-138	5	1550±30
Control	MM-138	-	1850±18
Control	HT-29	-	2800±25
PBS/2%FCS	-	-	3650±12

Discussion

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. Relaxivity enhancements were higher by a factor of 3 or 4 with Gd labeled DTPA protein conjugates than for Gd-DTPA alone. When Gd was conjugated with the 9.2.27 antibody by means of the chelator cDTPAa, a linear relation was observed between the reversal of T_1 relaxation time and the Gd concentration. The enhancing effect of Gd-DTPA-9.2.27 was compared with that of Gd-DTPA. Gd-DTPA-9.2.27 showed significant enhancement effect in melanoma cells compared to Gd-DTPA, controls, and $GdCl_3$.

Monoclonal antibodies labeled with gadolinium have been considered in order to effectively targeted the contrast agent to a tumor site. By using a melanoma-specific contrast agent such as Gd-cDTPAa-9.2.27, it may be possible to differentiate between melanoma, tissue damage and radiation injury.

References

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