Diagnostic Radiology

Australasian Radiology (2001) **45**, 298–304

In vitro *study of relationship between signal intensity and gadolinium-DTPA concentration at high magnetic field strength*

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SUMMARY

Although gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) has been used as a contrast material in MRI, it is known that the contrast enhancement effect is not uniform for high concentrations of Gd-DTPA. In order to evaluate the proper pulse sequences for dynamic MRI in aqueous solutions of Gd-DTPA, blood samples and melanoma cells, the signal intensity for several concentrations of Gd-DTPA were measured under inversion recovery (T_1-weighted) at high magnetic field strength (7.0 Tesla). For aqueous solutions of Gd-DTPA, signal intensity correlated linearly with the concentration of Gd-DTPA between 0 mmol/L and 4 mmol/L. Using blood and melanoma cells, signal intensity correlated non-linearly with the concentration of Gd-DTPA between 0 mmol/L and 1.5 mmol/L. For concentrations of more than 4 mmol/L in aqueous solutions of Gd-DTPA, 1 mmol/L in blood and 1.5 mmol/L in melanoma, signal intensity decreased with increased Gd-DTPA concentration.

Key words: *Gd-DTPA; magnetic resonance imaging; melanoma; signal intensity.*

INTRODUCTION

Magnetic resonance in clinical imaging and diagnosis provides diagnostic capabilities never previously enjoyed by the medical community. However, the quality of information obtained depends on the choice of instrument parameters. Before an MR examination can be designed, the operator must be aware of the consequences of the scanning parameter choices made, as they will determine the success or failure of the study.[1]

The contrast agent gadolinium–diethylene triamine pentaacetic acid (Gd-DTPA) reduces T_1 and T_2 relaxation times and creates very complex changes in the MRI signal.[2] Although Gd-DTPA has been used as a contrast agent in MRI, it is known that the contrast enhancement effect disappears if the concentration of Gd-DTPA increases beyond some levels.[3–6] Therefore, the determination of Gd-DTPA concentrations *in vitro* to provide optimal MRI signal intensity (SI) is vital to produce an image of the highest quality. On the other hand, it must be noted that the optimal pulse sequence will vary with field strength because of the frequency dependence of tissue T_1 relaxation times. A number of models, which describe the influence of the magnetic field strength on both T_1 and T_2 relaxation times, predict a significant increase in T_1 and a decrease in T_2 when using higher magnetic field strengths.[4,7] This decrease may result from the combination of susceptibility effects at elevated magnetic fields and diffusing of spins, resulting in a decreased value of T_2.

To investigate further whether SI with a contrast agent in blood is similar to SI with the contrast agents in soft tissue, melanoma cells were incubated in different concentrations of Gd-DTPA before MRI. These results were validated by experiments using magnetic field strengths of 1.9 Tesla for measurements of longitudinal relaxation rates in melanotic melanoma.[8]

The major goal of this work is to investigate the various MR parameters and their interdependence and to determine the optimal concentration of Gd-DTPA to enhance signal intensity

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Submitted 27 September 1999; resubmitted 12 April 2000; accepted 26 February 2001.

for *in vitro* studies at a high magnetic field strength. To this end, the SI of MRI in aqueous solutions of Gd-DTPA, aqueous solutions of sodium chloride in the presence of Gd-DTPA, blood and melanoma cells were measured with high magnetic field apparatus at 7.0 Tesla.

MATERIALS AND METHODS

Magnetic resonance images were obtained on a 300 MHz, 7.0 Tesla, Varian *UNITY* Plus (Varian Associated, Inc., Los Angeles, CA, USA) with a vertical magnet of bore size 89 mm (Oxford Instruments, Oxford, UK) using the saddle coil resonator. Five different types of samples were investigated in this work.

First, Gd-DTPA (Sigma Chemical Co., Aldrich, CA, USA) was dissolved in distilled water at different concentrations of 0.4–6.0 mmol/L. The volume of each Gd-DTPA was 1 mL, and each solution was put into a 5 mm nuclear magnetic resonance (NMR) test tube.

Second, a phantom containing seven tubes of aqueous solution of sodium chloride (5 mol/L) with different concentrations of Gd-DTPA was used. The concentrations of Gd-DTPA are the same as given earlier.

Third, different concentrations of sodium chloride solution (0, 0.3, 0.6, 1, 1.2, 2.5, 3.3 and 5 mol/L) were prepared in eight NMR test tubes filled with the same concentration of Gd-DTPA (4 mmol/L). These were grouped together for MRI.

Fifteen NMR test tubes with concentrations of 0–6 mmol/L Gd-DTPA in blood were then prepared. The SI measurements of these samples were carried out using the same experimental conditions as for the previous experiments (e.g. the same coil and pulse sequence).

Different solutions of Gd-DTPA (0–6 mmol/L) were added to melanoma cells (MM-138; Cancer Care Centre, St George Hospital, Sydney, NSW, Australia) and incubated. After 45 min, samples were placed into the centrifuge for 15 min at a speed of 1500 r.p.m. The cells were placed in fetal bovine serum and refrigerated for 12 h. The solution was separated from the cells and the cells inserted into 30-mm NMR test tubes for MRI.

In vitro T_1 and T_2 relaxation times measurements were made using an imaging probe in aqueous solutions (0–20 mmol/L of Gd-DTPA), blood samples and melanoma cells (0–10 mmol/L of Gd-DTPA). The T_1 and T_2 measurements were performed using inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, respectively. Relaxivities were then determined from the slope of the graph of relaxation rates as a function of Gd-DTPA concentrations.

All of these experiments were carried out using the same parameters. A conventional IR sequence was used and typical values were as follows: relaxation delay time (T_R) = 1000 ms, echo time (T_E) = 30 ms, inverse delay time (T_I) = 200 ms, slice thickness = 3 mm, field of view 25 mm × 25 mm, number of excitations (NEX) = 8, and matrix size = 512 × 28 or 512 × 256. A selective (sinc) RF pulse sequence with a flip angle of 180° was used for excitation. The voxel resolution was 2.1 mm × 2.1 mm × 2 mm.

In vitro SI measurements were performed by selecting voxels in the transfer image display. The brightness of any of the picture elements (pixels) is related directly to the intensity of the MR signal broadcasted by the corresponding voxel.[5] Five signal intensity measurements were chosen randomly for each voxel and an average of those was calculated as the SI. Signal intensities were normalized for a standard sample of 3 mmol/L of Gd-DTPA solution.

The MRI SI measurements for Gd-DTPA solutions were performed in two steps. First, the signal intensity was measured for each sample. In the second step, all of the samples, including seven NMR test tubes, were inserted in a 35 mm NMR test tube. The relationship between signal intensity and concentration of Gd-DTPA was also studied in two separate experiments. First, the contrast image of each sample was measured individually and second, all concentration samples were grouped together in a simple image.

RESULTS

Signal intensity and concentration of Gd-DTPA solution

In the case of sample experiments measured individually, the concentration of aqueous solution of Gd-DTPA and SI correlated linearly between up to 4 mmol/L under inversion recovery. Beyond 4 mmol/L, SI decreased with increased Gd-DTPA concentration. The plot of SI versus concentration of Gd-DTPA solutions is shown in Fig. 1a.

In the series of test tubes filled with different concentrations of Gd-DTPA, SI and concentration of Gd-DTPA correlated linearly up to 4 mmol/L under inversion recovery. Beyond 4 mmol/L, SI decreased with increased Gd-DTPA concentration. In this case, the plot of SI versus concentration of Gd-DTPA solutions is shown in Fig. 1b. According to these results, for both individual and collection tube samples together, Gd-DTPA concentrations up to 4 mmol/L should correlate linearly with SI of MRI using IR pulse sequence. As Fig. 1 shows, the shape of the calibration curves generated by each sample is similar to that for a series of samples in a simple phantom. R_1 and R_2 relaxivities for aqueous Gd-DTPA solutions were found to be 3.7/mmol per L/s and 4.8/mmol per L/s, respectively.

Signal intensity and concentration of Gd-DTPA solutions in the presence of sodium chloride

The effect of a biological fluid, such as sodium chloride, on the signal intensity for a water phantom containing different concentrations of Gd-DTPA and 5 mol/L NaCl was investigated. *In vitro* SI were measured by using transverse images of the samples. Figure 2 demonstrates the correlation between SI and aqueous solutions of sodium chloride with different

concentrations of Gd-DTPA. As can be seen from Fig. 2, SI correlated linearly with Gd-DTPA concentration in the range of less than 4 mmol/L. The overall intensity shape of this curve was approximately similar to that observed for Gd-DTPA.

Signal intensity and concentration of Gd-DTPA with different sodium chloride solutions

The variation in SI from a water phantom consisting of Gd-DTPA (4 mmol/L) with varying concentrations of sodium chloride is shown in Fig. 3. The resulting signal intensities are affected slightly by the concentration of sodium chloride. Regressions of signal intensities against Gd concentrations for *in vitro* measurements were always of good quality (Figs 2,3) with very low values of the mean square deviation. As can be seen from Fig. 3, SI slightly decreases with increased sodium chloride concentration; however, no significant changes were observed in the SI near isotonic solution (0.15 mmol/L of NaCl) when compared to water.

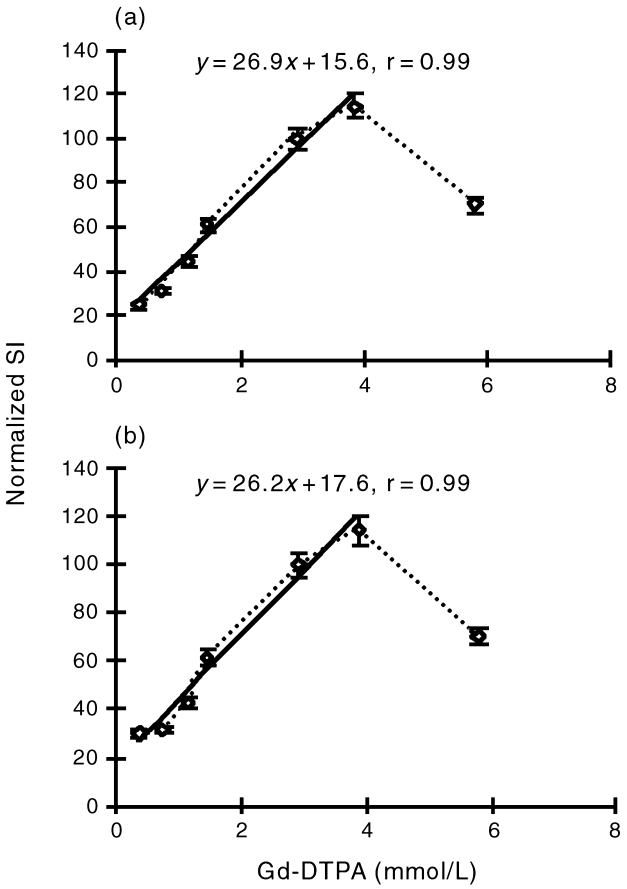

Fig. 1. Plot of normalized MRI signal intensity (SI). (a) Individual tubes and (b) collection tubes as a function of gadolinium–diethylene triamine penta-acetic acid (Gd-DTPA) concentration. (Peak response of signal intensity at 4 mmol/L Gd-DTPA concentration). The signal intensification at low concentrations of Gd-DTPA is due to T_1 shortening; the loss of signal at higher Gd-DTPA concentrations is due to T_2 shortening.

Signal intensity of blood samples with different Gd-DTPA concentrations

The signal enhancement properties of Gd-DTPA in blood samples were observed to rapidly increase non-linearly at a concentration of 1 mmol/L Gd-DTPA (Fig. 4). Above this concentration, SI decreased with increased Gd-DTPA concentration. In the concentration range studied, for low concentrations of Gd-DTPA (0–1 mmol/L in blood), a positive enhancement induced by the contrast agent was observed. T_1 relaxation time measurements were performed to confirm the validity of the results. T_1 values for blood containing 1 mmol/L of Gd-DTPA and blood without Gd-DTPA using an IR sequence were 200 ms and 1040 ms, respectively. R_1 and R_2 relaxivities for Gd-DTPA in blood were found to be 7.5/mmol per L/s and 10.7/mmol per L/s, respectively, and are reported for the first time.

The SI measurements of melanoma cells with different concentrations of Gd-DTPA (0–6 mmol/L) were measured. A reference water phantom was included in the imaging plane to correct for system variation, which is up to 25% less than that for an aqueous solution of Gd-DTPA for a given concentration. As Fig. 5 demonstrates, the enhancement ratio does not increase linearly with gadolinium concentration in melanoma. In addition, T_1 relaxation time measurements for aqueous solutions of Gd-DTPA (0.5 mmol/L) and melanoma cells with Gd-DTPA (0.5 mmol/L) were 1800 ms and 1100 ms, respectively. Table 1 shows T_1 relaxation time measurements of melanoma cells using other magnetic field strengths for comparison. As can be seen from this table, T_1 values for melanoma cells increase with increasing magnetic field strength. R_1 and R_2 relaxivities in melanoma cells were found to be 15.6/mmol per L/s and 27/mmol per L/s, respectively.

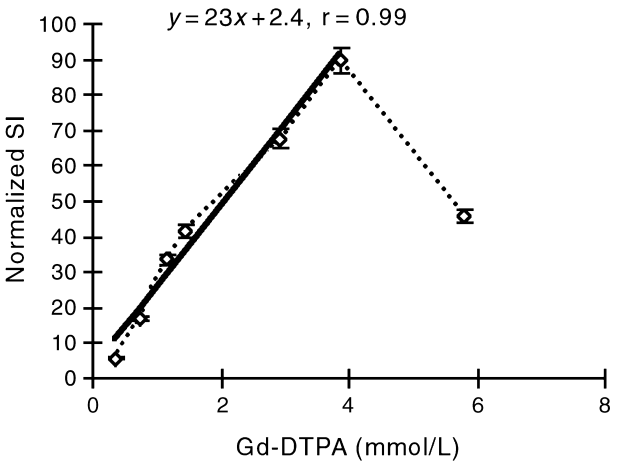

Fig. 2. Plot of normalized MRI signal intensity (SI) as a function of gadolinium–diethylene triamine pentaacetic acid (Gd-DTPA) concentration in 5 mol/L sodium chloride solution.

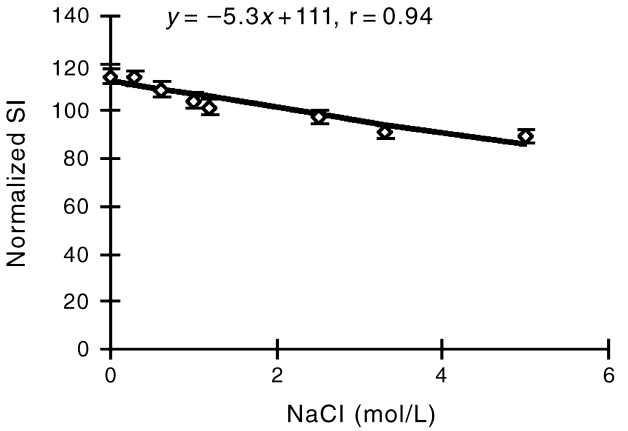

Fig. 3. Plot of normalized MRI signal intensity (SI) as a function concentration of sodium chloride solutions with constant gadolinium–diethylene triamine penta-acetic acid (Gd-DTPA) (4 mmol/L).

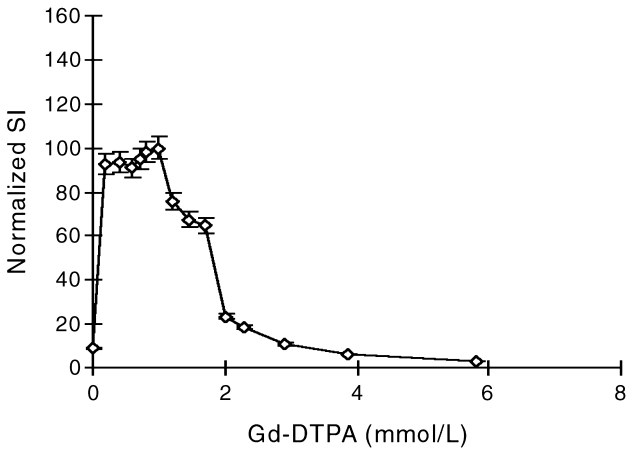

Fig. 4. Plot of normalized MRI signal intensity as a function of gadolinium–diethylene triamine penta-acetic acid (Gd-DTPA) in blood.

DISCUSSION

Signal intensity is the relative brightness level in an MRI. There are many different parameters that affect SI and the contrast obtained from various tissues in MR images.[6] Intrinsic parameters that are primarily responsible for the contrast obtainable in MR images are the proton densities and spin relaxation times. Extrinsic imaging factors that are under operator control are pulse sequence time, pulse timing parameters, paramagnetic contrast agent and radiofrequency pulse.

Gd-DTPA is one of only a few contrast agents available clinically for MRI at present.[1] Application of Gd-DTPA has made dynamic MRI of several organs and tissues possible, especially in cancer tissue at low magnetic field strengths.[9–11] However, the contrast enhancement effect of Gd-DTPA varies according to the pulse sequences,[12] magnetic field strength[13] and Gd-DTPA concentration.[3]

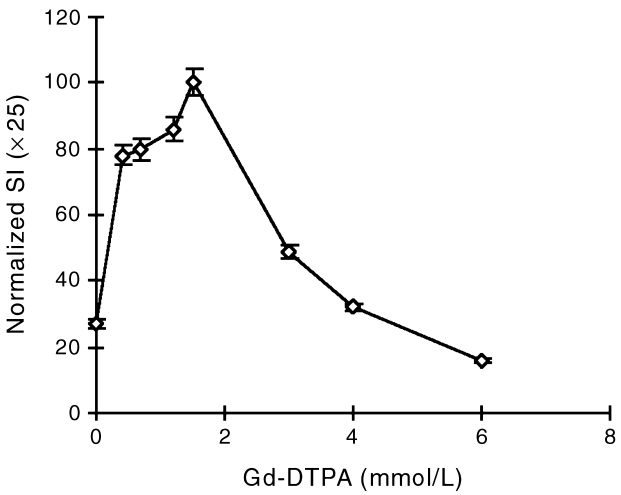

Fig. 5. Plot of normalized MRI signal intensity as a function of gadolinium–diethylene triamine penta-acetic acid (Gd-DTPA) concentration in melanoma cells.

Table 1. Longitudinal (T_1) relaxation measurements reported for *ex vivo* human melanoma cells with different magnetic field strengths[8,25]

Magnetic field strength (T)	T_1 relaxation (s)
1.4	1.0
1.9	1.2
2.7	1.3
7.0*	1.5*

*Measurement of T_1 in the present work.

Paramagnetic contrast agents, such as Gd-DTPA, may cause field-dependent changes in the nuclear spin relaxation rates and the effects on the gadolinium concentration are linear. Of course, the effect of data acquisition parameters, T_E and T_R in a spin-echo pulse sequence on the SI is given by the following equation:

$$(SI) = A \,.\, \exp(-T_E/T_2)\,[1 - \exp(-T_R/T_1)]$$

where A is a constant. Both T_R and T_E are a linear function of gadolinium concentration. The correlation curve of SI and gadolinium concentration will be a function of T_E and T_R; that is, a shorter T_R will move the turn-over seen to higher gadolinium concentrations as will a shorter T_E.

In CT or scintigraphy, the concentration of contrast medium or radioisotope almost correlates linearly with the CT number or radioactivity, whereas the concentration of Gd-DTPA does not correlate with SI in MRI,[13] the reason being that Gd-DTPA has both T_1 and T_2 shortening effects. At low Gd-DTPA concentrations, the T_1 shortening effect is dominant, and at high concentrations, the T_2 shortening effect is dominant.[3,6] In the latter condition, SI decreases with increasing Gd-DTPA concentration, as shown in this work. Signal plateaus at a high

Gd-DTPA concentration are caused by a complete relaxation between pulses, resulting in the sequence becoming proton density or PD-weighted instead of T1-weighted. However, the ideal contrast agent for positive-enhanced MRI should shorten the T_1 value but not change the T_2 value. Therefore, paramagnetic agents, such as Gd-DTPA, are known as T_1 agents resulting in MR T_1-weighted images.[14] For this reason, one of the important issues is the acceptable concentration of paramagnetic ion or contrast agents, especially the concentration of Gd-DTPA, under T_1-weighted images.

There have been several reports on tissue concentration of Gd-DTPA in animals at low magnetic field strength.[3,15] Using a 0.35 Tesla resist NMR unit, Brasch *et al.* showed that SI was enhanced with aqueous solutions of Gd-DTPA concentrations in the range of 0–5 mmol/L but then decreases with increasing Gd-DTPA concentrations under spin-echo sequences.[16] Takeda and his colleagues demonstrated the use of MRI to determine the *in vivo* SI versus different concentrations of Gd-DTPA at a low magnetic field strength (1.5 Tesla) in an aqueous solution of Gd-DTPA.[3] They showed that SI correlated linearly with the concentration of Gd-DTPA between 0 mmol/L and 3.0 mmol/L under spin-echo or gradient-echo sequences in rat kidney.

The optimal pulse sequence will vary with field strength because of the frequency dependence of T_1-relaxation times,[4,15] but the correlation between SI and Gd-DTPA concentration at high magnetic field strengths has not yet been investigated. Consequently, this report is the first to investigate *in vitro* relationships for machine parameters and pulse sequences at a high magnetic field strength of 7.0 Tesla.

The results of this work show that SI correlates linearly with Gd-DTPA concentrations from up to 4 mmol/L at a high magnetic field strength under IR pulse sequence (T_1-weighted) in aqueous solutions of Gd-DTPA. The results also indicate that a significantly improved fit to Gd-DTPA solutions in 4 mmol/L and sodium chloride at 4 mmol/L could be achieved by using T_1-weighted MRI (Fig. 3). The loss of SI at higher concentrations of Gd-DTPA is due to T_2-shortening but the signal intensification at low concentrations of Gd-DTPA is due to T_1-shortening. These imaging data provide more detailed information about aqueous solutions of Gd-DTPA than had been reported previously by Takeda *et al.* and Brasch *et al.*[3,16]

R_1 and R_2 relaxivities for aqueous Gd-DTPA solutions is consistent with the literature and supports the theory that relaxivity is dependent on magnetic field strength.[1,6]

The relaxation times are indirect measures of the interactions that occur between water molecules and other cell constituents.[17] Water molecules in biological tissue can be classified into two types: free and bound.[6] Free water is by far the more abundant and behaves like ordinary water in terms of its NMR properties. The bound fraction consists of water molecules that are bound rather firmly to the surface of molecules (cell membranes, protein, etc.) and are therefore far less mobile than their free counterparts. The limited mobility of bound water molecules causes a shorter T_1 (1–10 ms) relaxation time compared to water in the free state (2000 ms). In the present work the T_1 relaxation time was 2500 ms in free water.

As described earlier, blood has a shorter T_1 relaxation time and, hence, higher relaxivity values than that of water. For this reason, the SI in blood increases non-linearly with Gd-DTPA concentration to reach a maximum (1 mmol/L), and then decreases non-linearly as the concentration continues to increase. A similar decrease in SI has been observed with other paramagnetic agents; for example, ferric ions and nitroxide free radicals in high concentrations.[18,19] Signal enhancements in the present study were slightly larger than those reported by Siauve *et al.*,[20] in which the signal enhancements of carboxymethyl-dextran-Gd-DTPA in blood increased up to a concentration of 0.5 mmol/L at 4.7 Tesla. The correlation between SI and Gd-DTPA concentration in biological samples agrees with similar results obtained by Weinmann *et al.*[2]

T_1 relaxation values of tissues have been reported to vary with frequency and hence the applied magnetic field strength.[6] On the other hand, it must be noted that the optimal pulse sequence will vary with field strength because of the frequency dependence of tissue T_1 relaxation times. A number of models that describe the influence of magnetic field strength on both T_1 and T_2 relaxation times predict a significant increase in T_1 and a decrease in T_2 when using higher magnetic field strengths.[1,6] This result is consistent with the theory mentioned in the present article, which shows a higher T_1 relaxation time for melanoma cells at 7.0 Tesla compared to a lower magnetic field strength reported previously.[8,25]

Magnetic resonance imaging has significant potential for the detection and evaluation of melanoma.[8,21] The SI of malignant melanoma cells have been noted to differ from most other human neoplasms on MR images because melanomas often have shorter proton relaxation times.[22] Intratumoral melanin itself[23,24] and naturally occurring paramagnetic cations bound to melanin[25] have each been postulated as the major cause of shortened relaxation times in malignant melanomas. Atlas *et al.* have shown a correlation between shortening of T_1 and increasing melanin content.[8] Of course, a dipolar interaction can be associated with the presence of many other paramagnetic substances, including naturally occurring metals such as Fe^{3+}, Cu^{2+} or Mn^{2+}.[26] In particular, Fe^{3+} has been shown to bind to melanin excess paramagnetic and contribute significantly to the shortening of T_1 relaxation times.[25] For this reason, maximum SI versus Gd-DTPA in melanoma cells is lower than that for an aqueous solution. The aggregation of melanin into other macromolecular particles is another reason for the non-linear relationship between SI and Gd-DTPA in melanoma cells.

R_1 and R_2 relaxivities in melanoma cells were greater than that for aqueous Gd-DTPA solutions. Several mechanisms can

be suggested to explain the differences between the measured relaxivities of melanoma cells and aqueous solution in the presence of Gd-DTPA in *in vitro* conditions. These include a variation in the mechanism of rapid exchange of water molecules in aqueous Gd-DTPA solution and melanoma, macromolecular binding of contrast agent, and diffusion of protons through susceptibility gradients.

The results of the present study have confirmed that the binding of paramagnetic metal ions to the macromolecules of tissues has the largest effect on relaxation rates. These results are in reasonable agreement with the literature and indicate that the Gd-DTPA molecules pass through fenestrations in the membrane of abnormal cells or melanoma cells.[25]

Calibration curves are necessary for the adjustment of SI measurements and to provide an image of the highest quality. In the present report the measurements of SI versus Gd-DTPA concentration provides an insight into the MR enhancement ability of gadolinium. Although at this stage identification of the SI under *in vivo* conditions is not clear, it is possible that increases in SI are related to the T_1 shortening effect, as measurements show the T_1 relaxation times are 200 ms and 1040 ms for blood samples with and without 1 mmol/L of Gd-DTPA.

Differences between these data and previous experiments arise from different pulse sequences, magnetic field strengths and the method of measuring SI. But from the point of view of the correlation between SI and Gd-DTPA concentration, all results are similar.

Generally, the contrast agent is effective in enhancing T_1 and T_2 relaxation rates. Water and blood were used to estimate the error caused from using incorrect calibration curves. The calibration curves generated by direct signal measurements of water with different Gd-DTPA concentrations (0–6 mmol/L) by using inversion recovery pulse sequence are $y = 26.2x + 17.6$ and $y = 26.9x + 15.6$. On the other hand, the calibration curves for blood and melanoma cells with different Gd-DTPA concentrations obtained using inversion recovery pulse sequence show that a linear correlation does not exist between signal intensity and Gd-DTPA concentration.

CONCLUSIONS

In aqueous solutions of paramagnetic ions, a linear relationship between the paramagnetic ion concentration and relaxation rates is expected.[6] This relationship has been verified experimentally for aqueous Gd-DTPA solutions, Gd-DTPA solution in blood and in melanoma cells. These empirical data suggest that even in blood and soft tissues, T_1 and T_2 relaxation rates are proportional to the tissue gadolinium concentration.[3,13]

In addition, a number of models describing the influence of the magnetic field strength on both T_1 and T_2 relaxation times predicted a significant increase in T_1 and a decrease in T_2 when using higher magnetic field strengths.[4,6] This decrease was possibly due to the concentration of susceptibility effects at elevated magnetic fields and diffusion of spins, resulting in a decreased value for T_2.[5] Diffusion of spins through magnetic field variations introduces non-static inhomogeneities and leads to MR signal loss, even for pulse sequences that correct completely for static inhomogeneities. Magnetic susceptibility of samples for high concentrations of Gd-DTPA is responsible for T_2 shortening because of its comparatively large dipolar relaxation contribution, resulting in MR signal loss.

The present study has conclusively demonstrated that SI in aqueous solutions of Gd-DTPA are different to those for blood and melanoma. These results are similar to observations made by Tofts *et al.*[27] of phantom samples and tissue-equivalent materials with regard to relaxometry.

The results of the present study show several important findings. First, SI correlates with Gd-DTPA concentrations from 0 to 4 mmol/L in aqueous solutions of Gd-DTPA with high magnetic field apparatus. Second, SI is non-linear with Gd-DTPA concentration in blood within the range 0–6 mmol/L. Third, signal enhancement does not increase linearly with gadolinium concentration in melanoma cells in the range studied. Fourth, dynamic MRI (T_1-weighted) is possible under ordinary inversion recovery pulse sequence using a high magnetic field strength. It follows that images of high quality might be obtained if gadolinium concentrations within the compartments could be estimated.

The results of this work may help in the design of chemotherapeutic agents. The Gd-DTPA permeability study can provide information about tissue uptake of small chemotherapeutic agents for high resolution MR imaging studies. The paramagnetic contribution to the relaxation rates makes quantitative studies of the uptake of paramagnetic contrast agents possible. These findings may also be useful for MRI of melanoma cells at 7.0 Tesla.

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