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A Systematic Study on MR Contrast Agents for Constructing Specific Relaxation Times

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Abstract : The water proton relaxation rates increase linearly with concentrations of contrast agents, and could be expressed as a function of the concentrations. In this paper, we have investigated MR properties of two different contrast agents, $GdCl_3$ and $CoCl_2$. Relaxivity coefficients were calculated from individual contrast agent solutions, and used for predicting relaxation rates at mixtures of two contrast agents. From the experimental results, we have discussed the feasibility of constructing water solutions with the desired relaxation times using specific mixtures of contrast agents.

Keywords : Contrast agent, T_1 relaxation time, T_2 relaxation time, $GdCl_3$, $CoCl_2$, MRI

INTRODUCTION

Relaxation times are very important and sensitive probes for the determination of the structure and environment of matters in various magnetic resonance applications. In conventional NMR studies, relaxation times could be used in investigation of molecular dynamics, determination of molecular distances, and so on.[1-5] As well as spin density (i.e. concentration), relaxation times are one of the most dominant contrast-determining parameters in magnetic resonance (MR) imaging.[6,7] Therefore, it could be of great worth in NMR and MRI studies to control relaxation times suitable for the purposes. Paramagnetic compounds, as relaxation agents, have been widely used to control the relaxation times for

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enhancing efficiency of NMR and MRI experiments.[8-11] The electronic structures containing unpaired electrons which can fluctuate magnetic fields increase the relaxation rates associated with T_1 and T_2 of the interacting spins. These interactions also induce changes in the chemical shifts and linewidths of the spin signals, and the magnitudes of the changes are correlated with T_1 and T_2, respectively. For example, it is well known that the linewidth is inversely proportional to the transversal relaxation time T_2.

In this study, we have systematically investigated MR characteristics of two different types of contrast agents and their mixtures for constructing specific relaxation times. Gadolinium species, the paramagnetic materials frequently used for contrast agents, are known to accelerate both longitudinal and transversal relaxational rates (inversely proportional to T_1 and T_2 relaxation times, respectively) of water protons, but the effect of T_1 shortening is predominant.[7,12] On the other hand, Cobalt species have the strong effect on T_2 shortening but a relatively minimal effect on T_1.[8,13-15] For this reason, $GdCl_3$ and $CoCl_2$ were selected as relaxation agents in this study. For systematical analyses, relaxation times, chemical shifts and linewidths of water protons in the contrast agent solutions with various concentrations have been observed.[16,17] Additionally, to investigate the correlations between the two relaxation agents, those values for the mixture solutions of these relaxation agents were also measured.[18] Based on these experimental results, we have discussed the feasibility of constructing water solutions with the desired relaxation times using specific mixtures of contrast agents.

EXPERIMENTAL

NMR solvents and MR contrast agents ($GdCl_3 \cdot 6H_2O$ and $CoCl_2 \cdot 6H_2O$) used in this study were purchased from Sigma-Aldrich Chemical Co. Inc. All experiments were performed on a 600 MHz Bruker Avance DMX600 NMR spectrometer with a TXI probe at room temperature. To prevent the radiation damping effects by condensed water proton signals, each sample solution (~0.10 mL) was put in a central capillary tubes (inner diameter = 1.5 mm),[19-22] and the capillary was then placed within a regular 5-mm NMR tube

that contains 99% D_2O solvent. The signal from the outer water (99% D_2O) was used as an external standard to measure changes in chemical shifts and linewidths depending on the concentration of contrast agents. Samples with various concentrations (0.5 mM ~ 2.0 mM) of each contrast agent and/or their composites (16 samples) were prepared for NMR experiments. Inversion recovery and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences were employed for measurements of T_1 and T_2 relaxation times of contrast agent solutions, respectively.[23,24]

Relaxivity coefficients, r_1 and r_2, were obtained from the linear regression analyses of $1/T_1$ and $1/T_2$ versus contrast agent concentrations. For example, $r_{1,Gd}$ (in $mM^{-1}\cdot s^{-1}$) was calculated as the slope of the fitted line for $1/T_1$ data acquired at different $GaCl_3$ concentrations. The calculations for the other coefficients could be accomplished by similar plots. If there is no mutual interaction between $GaCl_3$ and $CoCl_2$ contrast agents, the relaxivity rates R_1 and R_2 could be expressed as a simple function of each contrast agent concentration. As shown in Eq. (1), the overall relaxivity rate of a composite contrast agent solution is a linear combination of the relaxivity rate of free water and the additional relaxivity contribution from each contrast agent.

$$R_1 = R_{1,free} + R_{1,CA} = \left(\frac{1}{T_{1,free}}\right) + r_1 \cdot [CA] = \left(\frac{1}{T_{1,free}}\right) + r_{1,Gd} \cdot [Gd] + r_{1,Co} \cdot [Co]$$

$$R_2 = R_{2,free} + R_{2,CA} = \left(\frac{1}{T_{2,free}}\right) + r_2 \cdot [CA] = \left(\frac{1}{T_{2,free}}\right) + r_{2,Gd} \cdot [Gd] + r_{2,Co} \cdot [Co] \quad (1)$$

Where $T_{1,free}$ and $T_{2,free}$ are the longitudinal and transverse relaxation time of the contrast-agent-free water protons, respectively. [Gd] and [Co] represent the concentration in milimolar of $GdCl_3\cdot 6H_2O$ and $CoCl_2\cdot 6H_2O$ in the sample solution. $r_{1,Gd}$, $r_{1,Co}$, $r_{2,Gd}$ and $r_{2,Co}$ correspond to the relaxivity coefficients obtained from T_1 and T_2 experiments for single contrast agent solutions.

RESULTS AND DISCUSSION

Figure 1 showed the chemical shifts and linewidths changes of ^{1}H NMR signals from water protons depending on the concentration of each contrast agent. As expected, paramagnetic lanthanide Gd^{3+} ions (from $GdCl_3$) induced large changes in chemical shifts (~0.12 ppm/mM) due to the specific electronic structure having the maximum number of unpaired electrons. Seven unpaired electrons arrange symmetrically around the metal and interact with nearby water molecules. Co^{2+} ions (from $CoCl_2$) also could create changes in chemical shifts, but the effect was nearly half of Gd^{3+} ions. For the samples with the mixtures of two contrast agents, as shown in Table 1, the chemical shifts changes reflected simply the individual concentration of each contrast agent. These results implied that the chemical shift changes could be expected by the contrast agent concentrations, and vice versa.

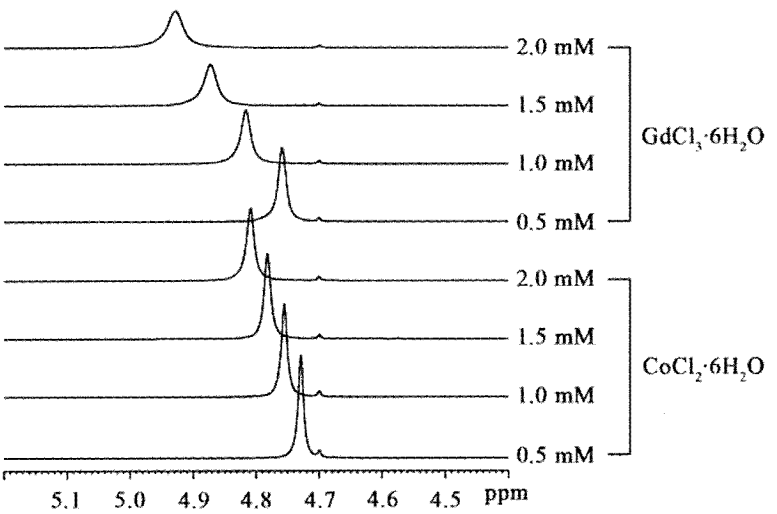

Figure 1. Variations of the chemical shift and linewidth of water proton NMR peaks with different concentrations of contrast agents (small peaks at 4.7 ppm correspond to water proton signals from pure H_2O).

Table 1. Chemical shifts (ppm) of water proton NMR peaks of composite contrast agent solutions.

	Co 0.0 mM	Co 0.5 mM	Co 1.0 mM	Co 1.5 mM	Co 2.0 mM
Gd 0.0 mM	4.70	4.73	4.76	4.78	4.81
Gd 0.5 mM	4.76	4.79	4.81	4.84	4.87
Gd 1.0 mM	4.82	4.84	4.87	4.90	4.92
Gd 1.5 mM	4.87	4.90	4.93	4.95	4.98
Gd 2.0 mM	4.93	4.96	4.98	5.01	5.04

Linewidths of NMR signals were also widened with the increment of contrast agent concentrations. However, the linearity in the changes compared with chemical shifts was reduced by additional line broadening effects, such as different field inhomogeneity in each sample.

Relaxivity coefficients (r_1, r_2) of $GdCl_3$ and $CoCl_2$ were calculated from the slope of $1/T_1$ and $1/T_2$ graphs (Fig. 2) and are listed in Table 2. Similar to the chemical shifts variation results, $GdCl_3$ has large relaxivity coefficients both in longitudinal and transverse relaxation processes. Additionally, the r_2/r_1 ratio of 1.4 means that $GdCl_3$ should act as a good T_1 contrast agent for positively contrasted MRI applications.[25] On the other hand, $CoCl_3$ showed a predominant relaxivity effect of r_2 related with transverse relaxation ($1/T_2$) of water proton signals. Since $CoCl_3$ would not disturb the longitudinal relaxation time T_1 (resulting a very small relaxivity coefficient $r_{1,Co}$), the r_2/r_1 ratio becomes very large (>40) enough for negatively contrasted MRI applications (as T_2-contrast agents).

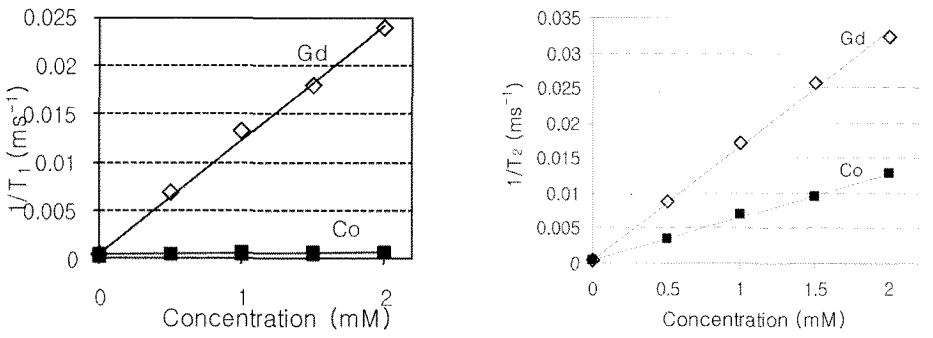

Figure 2. Relaxivity rates $1/T_1$ (left) and $1/T_2$ (right) at different concentrations of $GdCl_3$ (open diamond) and $CoCl_2$ (filled rectangle) to calculate corresponding relaxivity coefficients (r_1 and r_2).

Table 2. Relaxivity coefficients (r_1, r_2) in mM^{-1} s^{-1} for water proton signals in $GdCl_3$ and $CoCl_2$ solutions.

Contrast agents	r_1 (mM^{-1} s^{-1})	r_2 (mM^{-1} s^{-1})	r_2/r_1
$GdCl_3$	11.9	16.3	1.4
$CoCl_2$	0.14	6.2	44.3

Table 3. Comparison of experimental and calculated T_1 relaxation times of water protons at different composite contrast agent solutions.

T_1 (ms)	Co 0.5 mM		Co 1.0 mM		Co 1.5 mM		Co 2.0 mM	
	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.
Gd 0.5 mM	147 ± 3	156	146 ± 2	155	145 ± 2	154	143 ± 4	151
Gd 1.0 mM	75 ± 3	81	75 ± 2	81	74 ± 3	80	73 ± 4	79
Gd 1.5 mM	55 ± 3	55	54 ± 3	55	54 ± 2	54	53 ± 2	54
Gd 2.0 mM	42 ± 2	41	41 ± 1	41	41 ± 1	41	40 ± 2	41

Table 4. Comparison of experimental and calculated T_2 relaxation times of water protons at different composite contrast agent solutions.

T_2 (ms)	Co 0.5 mM		Co 1.0 mM		Co 1.5 mM		Co 2.0 mM	
	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.
Gd 0.5 mM	86 ± 2	85	68 ± 3	67	57 ± 2	56	49 ± 2	48
Gd 1.0 mM	49 ± 1	50	43 ± 2	44	37 ± 2	38	33 ± 2	34
Gd 1.5 mM	38 ± 5	35	35 ± 3	32	32 ± 2	29	30 ± 1	27
Gd 2.0 mM	30 ± 4	28	29 ± 1	25	27 ± 2	23	26 ± 2	22

The relaxivity coefficients obtained from the analysis of the relaxation experiments, as depicted in Fig. 2, were used to predict the R_1 and R_2 relaxation rates (and/or relaxation times T_1 and T_2) of water protons in composite contrast agent solutions. All relaxation rates were calculated from Eq. (1), and then compared with the experimental results (see Tables 3 and 4).

Relatively large differences (~ 8 %) in T_1 values occurred at the composite contrast agent solutions with 0.5 mM and 1.0 mM $GdCl_3$, and it could be expected from the linear regression plot for calculating the relaxivity coefficient $r_{1,Gd}$ in Fig. 2. On the contrary to T_1, the experimental T_2 values of solutions with high concentration of contrast agents (> 1.0

mM) were larger than the calculated values (~ 20 %). This could be the results of mutual interactions between Gd^{3+} and Co^{2+} ions in water solutions.

To test the feasibility of constructing desired relaxation times using mixtures of contrast agents, we have made three samples being expected to have T_1 of 100 ~140 ms and T_2 of 40 ~ 60 ms from Eq. (1). As shown in Table 5, the errors in T_1 and T_2 between the calculated and experimental values were less than 5 % and 10%, respectively.

Table 5. Comparison of experimental and calculated T_1 and T_2 relaxation times for specific mixtures of contrast agents.

Sample	Contrast Agent		Calculated Values		Experimental Values	
	$GdCl_3$	$CoCl_3$	T_1	T_2	T_1	T_2
A	0.80 mM	2.00 mM	98	39	98 ± 2	44 ± 1
B	0.80 mM	0.50 mM	100	60	98 ± 2	64 ± 2
C	0.55 mM	1.20 mM	141	59	141 ± 3	66 ± 2

Although there are some of discrepancies between the calculated and experimental values, we were able to see a feasibility using composite contrast agents to create specific relaxation rates of samples. This property can be applied to contrast enhanced MRI experiments such as cellular labeling.

In summary, we have presented relaxational properties of two different types of MR contrast agents ($GdCl_3$ and $CoCl_2$) and their mixtures. At different concentrations of contrast agents, T_1 and T_2 relaxation times and chemical shifts of water protons were acquired. The chemical shifts changes were proportional to the individual concentration of each contrast agent. Relaxivity coefficients (r_1 and r_2) were calculated from the slope of $1/T_1$ and $1/T_2$ graphs, and were used to predict relaxation rates R_1 and R_2. The predicted T_1 and T_2 relaxation times were fairly consistent with the experimental values. All these experimental results show the feasibility of constructing desired relaxation times using mixtures of contrast agents.

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