

^{17}O NMR Study On Water Exchange Rate of Paramagnetic Contrast Agents

Yongmin Chang^{1,3}, Sung Wook Hong², Moon Jung Hwang², Il Soo Rhee², Duk-Sik Kang^{1,3}

Purpose : The water exchange rate between bulk water and bound water is an important parameter in deciding the efficiency of paramagnetic contrast agents. In this study, we evaluated the water exchange rates of various Gd-chelates using oxygen-17 NMR technique.

Material and Methods : The samples (Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA, Gd-EOB-DTPA) were prepared by mixing 5% ^{17}O -enriched water (Isotech, USA). The pH of the samples was adjusted to physiological value (pH = 7.0) by buffer solution. The variable temperature ^{17}O -NMR measurements were performed using Bruker-600 (14.1 T, 81.3 MHz) spectrometer. Bruker VT-1000 temperature control units were used to stabilize the temperature. The ^{17}O spin-spin relaxation times (T2) were measured using Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with 24 echo trains. The variable temperature T2 relaxation data were then fitted into Solomon-Bloembergen equations using least square fit algorithm to estimate the water exchange times. .

Results : From the measured ^{17}O -NMR relaxation rates, the determined water exchange rates at 300K are : 0.42 μs for Gd-DTPA, 1.99 μs for Gd-DTPA-BMA, 0.27 μs for Gd-DOTA, and 0.11 μs for Gd-EOB-DTPA. The Gd-DTPA-BMA showed slowest exchange whereas Gd-EOB-DTPA had fastest water exchange rate. In addition, it was found that the water exchange rates (τ_m) of all samples had exponential temperature dependence with different decay constant.

Conclusion : ^{17}O -NMR relaxation rate measurements, when combined with variable temperature technique, provide a solid tool for studying water exchange rate, which is very important in investigating the detailed mechanism of relaxation enhancement effect of the paramagnetic contrast agents.

Index words : Paramagnetic,
Contrast Agent,
Water exchange,
NMR,
Relaxation

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¹Department of Diagnostic Radiology, College of Medicine,

²Department of Physics, College of Natural Science,

³The Institute of Medical Imaging, College of Medicine, Kyungpook National University

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Address reprint requests to : Yongmin Chang, Ph.D. Department of Diagnostic Radiology, College of Medicine,

Kyungpook National University and Hospital, 50 Samduk-dong, Taegu 700-412, Korea.

Tel. 82-53-420-5471, Fax. 82-53-422-2677

Introduction

Stable complexes of the gadolinium (Gd) ion in aqueous solution are often used as actual contrast agents in clinical magnetic resonance imaging (MRI) (1–3). In order to produce relaxation enhancement, the complex must modify significantly the proton relaxation rates of water in its vicinity. Around a paramagnetic ion, the bulk water proton relaxation rates are enhanced due either to long-range interaction or to short-range interactions mediated by exchange with bound water molecules (4–5). This short-range interaction, which is called as inner sphere relaxation, has the largest contribution to the relaxation enhancement. The inner sphere effect is determined by three correlation times : the time for rotation of the complex (τ_R), the water exchange time of a water proton in the inner coordination sphere (τ_m), and the longitudinal electronic relaxation time (T_{1e}). Especially, the water exchange rate (τ_m) has a dual importance in relaxation enhancement : it can contribute to the overall correlation time (τ_C) and it modulates the efficiency of the chemical exchange of water molecules sampling the paramagnetic ion. In this study, we investigated the water exchange rates of the most widely used paramagnetic contrast agents using variable temperature ^{17}O -NMR technique. The technique, which measures the exchange rate of the oxygen atom of the water, has been known to provide information on the chemical exchange reaction mechanism (6–7). In addition, we also obtained the kinetic parameters including the activation enthalpy, and the parameters governing electronic relaxation.

Materials and Methods

Sample Preparation

For ^{17}O -NMR measurements, three most widely used Gd-based contrast agents (Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA) and liver-specific MR agent, Gd-EOB-DTPA were prepared by mixing 5% ^{17}O -enriched water (Isotech, USA). The pH of the samples was adjusted to physiological value (pH = 7.0) after filtration.

NMR Measurement

Variable temperature ^{17}O -NMR measurements were performed using Bruker-600 (14.1 T, 81.3 MHz) spec-

trometer. Bruker VT-1000 temperature control units were used to stabilize the temperature in the range of 270 - 330K. The samples were sealed in glass spheres, fitting into 5-mm NMR tubes, in order to eliminate susceptibility corrections to the chemical shift. All transverse relaxation rates, $1/T_2$, were measured by the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. The 24 echo trains were used in this study. The measured T2 relaxation data were then interpreted using the Solomon-Bloembergen-Morgan (SBM) theory as well as the equations given by Swift and Connick (8–10).

Theoretical Model

The Swift and Connick equation based on SBM theory is given by

$$\frac{1}{T_{2r}} = \frac{1}{P_m} = \left[\frac{1}{T_2} - \frac{1}{T_{2A}} \right] = \frac{1}{\tau_m} \frac{T_{2m}^2 + \tau_m^{-1} + T_m^{-1} + \Delta\omega_m^2}{(\tau_m^{-1} + T_{2m}^{-1})^2 + \Delta\omega_m^2} + \frac{1}{T_{2OS}} \quad [1]$$

where, $1/T_{2m}$ is relaxation rate in the bound water and $1/T_{2A}$ is the relaxation rate of water reference. $\Delta\omega_m$ is the chemical shift difference between bound water and bulk water in the absence of a paramagnetic interaction with the bulk water. P_m is the mole fraction of bound water and τ_m is the residence time of water molecules in the inner coordination sphere. T_{2OS} is the total outer sphere contributions to the reduced relaxation rate. Since the outer sphere contribution ($1/T_{2OS}$) and the chemical shift ($\Delta\omega_m$) are relatively small, we can neglect these two terms. The transverse relaxation of an ^{17}O nucleus of bound water can be expressed as a sum of three relaxation mechanisms : (1) the relaxation due to interaction of the electric quadrupole moment with electric field gradients, (2) the scalar interaction of the nuclear spin with the electron spin, and (3) the dipole-dipole interaction of the nuclear spin with the electron spin. In the case of paramagnetic ion, the scalar contribution is by far the most important and it is given to an excellent approximation by

$$\frac{1}{T_{2m}} = \frac{S + (S + 1)}{3} \left(\frac{A}{h} \right) \tau_{s1} \quad [2]$$

where $1/\tau_{s1} = 1/\tau_m + 1/T_{1e}$. From the EPR study of

Gd-complex, it was found that the longitudinal electronic relaxation (T_{1e}) is due to modulation of a static or transient zero-field splitting (ZFS). Based on this observation, it was shown that the longitudinal electronic relaxation (T_{1e}) should, to an excellent approximation, be a single exponential that can be described by the analytical expression of McLachlan

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25} \Delta^2 \tau_v [4S(S+1)-3] \left(\frac{1}{1+\omega_s^2 \tau_v^2} + \frac{4}{1+4\omega_s^2 \tau_v^2} \right) \quad [3]$$

where ω_s is the electron Larmor frequency, Δ^2 is the trace of the square of the ZFS tensor, and τ_v is the correlation time for modulation of the ZFS. The modulation of the ZFS may be due either to rotation of the complex or to the lifetime of transient distortion, and we assume that the correlation time has Arrhenius behavior

$$\tau_v = \tau_v^{273.15} \exp [E_v / R (1/T - 1/273.15)] \quad [4]$$

where $\tau_v^{273.15}$ is the correlation time at 273.15 K and E_v is the activation energy for the process. The exchange rate (k_{ex}) of water molecules in the inner sphere is assumed to obey the Eyring equation

$$\frac{1}{\tau_m} = \frac{k_B T}{h} \exp \left[\frac{\Delta S}{R} - \frac{\Delta H}{RT} \right] \quad [5]$$

where ΔS and ΔH are the entropy and enthalpy of activation for the exchange process. Using equations (1) – (5), the simultaneous least-square fit of the temperature variable T2 relaxation data will result in six fitted parameters, ΔS , ΔH , Δ , $\tau_v^{273.15}$, E_v , and A.

Results and Discussion

The temperature dependence of the transverse relaxation rates of the Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA, and Gd-EOB-DTPA solutions are shown in Figure 1. For the analysis, a combined nonlinear least-square treatment of all corresponding data was performed using eqs [1] – [5]. A least-square fit of these data allows an estimation of the parameters. In a first approximation we neglected the contribution from the outer-sphere interaction with the Gd³⁺. The adjusted parameters are ΔS , ΔH , Δ , $\tau_v^{273.15}$, E_v , and A. The last three parameters defining the electronic relaxation time (T_{1e}) are strongly correlated, leading to potentially relative statistical errors. The results are given in Table I and the calculated curves, basing on estimated parameters, are shown as solid lines in Figure 1. There is a good agreement between the adjusted parameters and the experiment. The water exchange rates at 300 K, which deter-

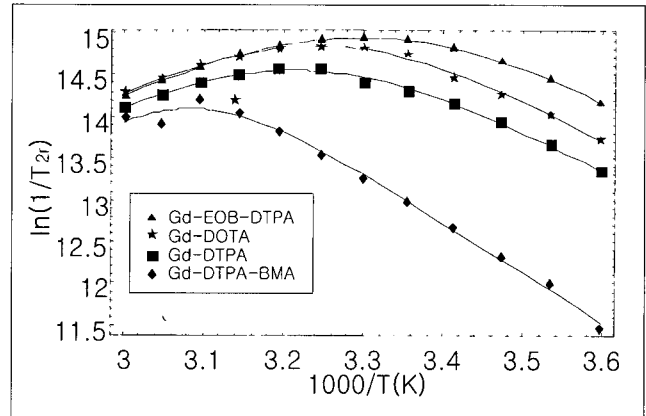


Fig. 1. Variable temperature ¹⁷O NMR transverse relaxation rates (S-1) data for Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA, and Gd-EOB-DTPA. The lines are the result of a non-linear least-square fit of these data using eqs [1] – [5].

Table I. Kinetic Parameters Obtained from Least-square Fits of ¹⁷O NMR Transverse Relaxation Data for Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA, and Gd-EOB-DTPA.

	Gd-DTPA-BMA	Gd-DOTA	Gd-DTPA	Gd-EOB-DTPA
ΔS (J K mol ⁻¹)	23.11 ± 28.1	18.043	6.3436	51.204
ΔH (kJ mol ⁻¹)	47678 ± 8.21	41158	38758	48865
Δ (× 109 s ⁻¹)	5.86453 ± 8.78	1.05291	0.990337	0.623819
$\tau_v^{273.15}$ (μs)	4.5828 ± 27.20	0.12662	0.19924	2.5469(ns)
E_v (kJ mol ⁻¹)	68956 ± 130230	30111	35581	5897.1
A / \hbar (× 10 ⁷ rad s ⁻¹)	-8.8009 ± 1014	-3.3891	-2.6358	-5.6035

mined from the adjusted parameters, are 0.42 μs for Gd-DTPA, 0.27 μs for Gd-DOTA, 1.99 μs for Gd-DTPA-BMA, and 0.11 μs for Gd-EOB-DTPA. Our results also showed that the water exchange times of Gd-complexes are temperature dependent (Figure 2). Whereas all agents showed exponential temperature behavior, the temperature constants (T_{const}), which defined as $\exp(-T/T_{\text{const}}) = \exp(-1)$, are different. Among Gd-complexes, Gd-DTPA-BMA has the longest T_{const} and shows strongest temperature dependence. For the rest of Gd-complexes, the temperature dependence of the water exchange rate is much weaker than Gd-DTPA-BMA and the differences in τ_m are shown only at lower temperature ranges. That is, at body temperature (310 K), three Gd-complexes have almost same water exchange rate.

It is also interesting to consider the kinetic parameters we obtained. First, all Gd-complexes have positive activation entropy. Since negative activation entropy, which was shown in previous study (7), means associatively activated water exchange, the positive entropy of all agents indicates dissociatively activated water exchange. This can be understood if the Gd-complexes studied can accommodate only one inner sphere water molecule. That is, the entering water molecule can be coordinated only on the site of the leaving water molecule and so the process is dissociative. Second, among Gd-complexes, Gd-DTPA has smallest activation entropy and activation enthalpy. In addition, since Gd-DT-

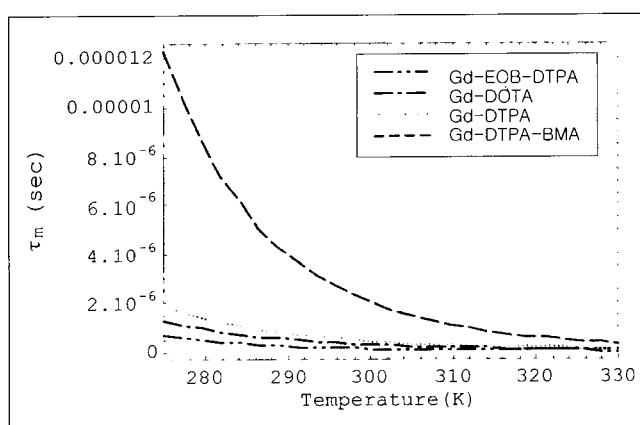


Fig. 2. Temperature dependence of water exchange times for Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA, and Gd-EOB-DTPA. Among Gd-complexes, Gd-DTPA-BMA shows strongest temperature dependence. For others, the temperature dependence of the water exchange rate is much weaker than Gd-DTPA-BMA and the differences in τ_m are shown only at lower temperature ranges.

PA requires least activation energy, the water exchange between bulk water and bound water of Gd-DTPA would be relatively easy. That is, Gd-DTPA is expected to have short water exchange time as shown in our result.

The oxygen-17 NMR is not only way to study water exchange between bound water of paramagnetic ion and bulk water. The NMRD (Nuclear Magnetic Relaxation Dispersion) study of proton can also provide the information by fitting NMRD data (11–12). However, in analysis of NMRD data, there are several correlation times involved and it is impossible to measure τ_m independently. In addition, the proton and the oxygen have different interaction with the electron spin of the Gd ion. That is, while the oxygen in the bound water interacts mainly via scalar interaction with the electron spin of the Gd ion, the proton has a much higher contribution from dipole-dipole interaction with the electron spin.

Conclusion

^{17}O NMR relaxation rate measurements at several different temperatures provide a powerful tool for studying water exchange in aqueous solutions of paramagnetic Gd-complexes. The measured water exchange rates at 300K are 0.42 μs for Gd-DTPA, 0.27 μs for Gd-DOTA, 1.99 μs for Gd-DTPA-BMA, and 0.11 μs for Gd-EOB-DTPA respectively. Also, the kinetic parameters for water exchange on all Gd-complexes, which investigated in this study, indicate dissociative water exchange mechanism. In conclusion, the exchange of water molecules between the inner sphere (water binding site) of Gd and the bulk water is of high importance in proton relaxation enhancement by paramagnetic contrast agent, since the paramagnetic effect on bound water is transferred to the bulk water (and to the tissue) by this exchange process.

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¹⁷O NMR 기법을 이용한 상자성 자기공명조영제의 물분자 교환에 관한 연구

¹경북대학교 의과대학 진단방사선과학교실,

²경북대학교 자연대학 물리학과

³경북대학교 의학영상연구소

장용민^{1,3} · 홍성욱² · 황문정² · 이일수² · 강덕식^{1,3}

목적 : 상자성 자기공명조영제의 효율을 결정하는데는 상자성물질의 물분자 결합위치에 구속되어 있는 물분자와 자유 물 분자사이의 물분자 교환율이 매우 중요한 역할을 담당한다. 따라서 본 연구에서는 ¹⁷O-NMR 기법을 사용하여 현재 상용화 되어 있는 Gd 자기공명조영제 및 최근 간특이성 자기공명조영제로 제안되고 있는 Gd-EOB-DTPA의 물분자 교환율을 측정하고자 하였다.

대상 및 방법 : 본 연구에 사용된 조영제는 Gd-DTPA, Gd-DTPA-BMA, Gd-DOTA, Gd-EOB-DTPA 이며 여기에 Isotech 사의 5% ¹⁷O 로 치환된 증류수를 혼합하여 사용하였다. 결과적인 시료의 pH는 buffer 용액을 사용하여 pH = 7 로 고정하였으며 다양한 온도에서 Bruker-600 (14.1 T, 81.3 MHz) 모델의 NMR 장비를 사용하여 측정하였다. 에코열 24개의 Carr-Purcell-Meiboom-Gill (CPMG) 펄스 시퀀스를 사용하여 ¹⁷O의 스핀-스핀 이완시간(T2)을 측정하고 이렇게 얻어진 T2 데이터는 최소자승법을 이용하여 Solomon-Bloembergen 방정식에 fitting 시켜서 최종적으로 각 조영제의 물분자 교환율을 계산하였다.

결과 : 측정된 각 조영제의 물분자 교환시간은 300K의 온도에서는 Gd-DTPA의 경우 0.42 μ s, Gd-DTPA-BMA의 경우 1.99 μ s, Gd-DOTA의 경우 0.27 μ s, Gd-EOB-DTPA의 경우 0.11 μ s로 나타났으며 이러한 물분자 교환시간은 온도에 따라 변화함을 알았다. 물분자 교환시간의 온도 의존성은 모든 조영제에서 지수함수의 형태로 나타났으나 조영제에 따라 온도가 올라감에 따라 물분자 교환시간이 감소하는 감소율에서는 차이를 나타내었다.

결론 : 상자성 조영제의 relaxation enhancement 기전을 이해하는데는 물분자 교환율에 대한 정보가 매우 중요하며 이러한 물분자 교환율을 정확히 측정하는데는 ¹⁷O-NMR 기법이 매우 유용함을 알 수 있었다.

통신저자 : 장용민, 대구시 중구 삼덕동 2가 50, 경북대학교 의과대학 방사선과학교실
Tel. 82-53-420-5471 Fax. 82-53-422-2677