● *Original Contribution*

MAGNETIC FIELD DEPENDENCE OF SPIN-LATTICE RELAXATION ENHANCEMENT USING PIPERIDINYL NITROXYL SPIN-LABELS

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We examined the magnetic resonance properties of 12 paramagnetic piperidinyl nitroxyls in water and plasma
solutions. Paramagnetic contributions to proton relaxation times were measured using 10.7 and 100 MHz
spectrometers. Proton relaxation enhancement from nitroxyls increased with ascending molecular weight, in plasma
solutions versus equimolar aqueous solutions, and with measurements at 10.7 MHz compared to 100 MHz.
Relaxation rates were observed to approximately double at 10.7 MHz compared to 100 MHz and from water to
plasma solutions. The data indicate that proton spin-lattice relaxation enhancement is magnetic field-dependent, and
increases using nitroxyls of large molecular weight and with chemical substitutents that increase the microviscosity of
solvent water molecules. The development of nitroxyls for diagnostic MRI will be aided by understanding these in
vitro physical characteristics and trends.

Keywords: Nitroxyl spin labels, Magnetic resonance contrast media, Field-dependent relaxation

INTRODUCTION

Magnetic resonance imaging (MRI) has exciting potential as a diagnostic tool useful in clinical imaging of the human body.[7,13,19,51] Contrast agents may augment diagnostic information from MRI. Numerous contrast agents have been proposed including lasix,[2] perfluorocarbon emulsions,[15] dilute oral iron solutions for gastrointestinal contrast,[46] and rare-earth complexes;[14,45] the last two categories of compounds are termed paramagnetic because they possess an electron with an unpaired spin.[25,34,42]

The strength and time decay of the MRI signal depends on a tissue's hydrogen density, intrinsic longitudinal relaxation time (T_1), intrinsic transverse relaxation time (T_2),[30] and the translational self-diffusion coefficient.[48,49] These relaxation times are influenced by the microscopic magnetic environment surrounding each proton. Because the combination of these properties varies among tissues, excellent inherent contrast in MRI is obtainable among bodily organs.

MRI is exquisitely sensitive to the detection of disease, but may be rather poor in its ability to specifically diagnose an abnormality.[34] Diagnostic specificity may be improved with tissue-specific paramagnetic pharmaceuticals. Nitroxyl spin labels (NSLs) have potential as paramagnetic agents for MRI because of a chemically stable unpaired electron. When NSL are placed in a magnetic field, the electron aligns with the field; as a much stronger magnetic dipole than unpaired protons, the unpaired electron alters the local magnetic field experienced by protons around it, reducing T_1 and T_2 relaxation times (increasing relaxation rates, $1/T_1$, $1/T_2$). This property is termed paramagnetic proton relaxation enhancement.

NSL are synthetic organic free radicals and are, as a class, potentially useful paramagnetic contrast agents for MRI. Like other paramagnetic substances, NSLs increase T_1 and T_2 relaxation rates of hydrogen nuclei, thus altering signal intensity. We report data that compare efficiency of proton relaxation enhancement

using a series of piperidinyl NSLs differing in size and substituents.

The experimental design compares proton spin-lattice relaxation in water and plasma at two widely different, but clinically relevant, magnetic field strengths: 0.25T and 2.3T. These two field strengths approximate "low-field" and "high-field" human MRI systems currently in use. We report a range of from 1.5- to 3.5- fold decrease in NSL-induced spin lattice relaxation rate enhancement comparing low-field to high-field results.

These in vitro data from multiple concentrations of each NSL provide a foundation for in vivo testing of the more successful relaxing agents of these NSL, and indicate a trend of magnetic-field dependence of the proton spin-lattice relaxation enhancement using NSLs.

MATERIALS AND METHODS

Chemicals:

Twelve piperidinyl NSL compounds were either purchased commercially (Eastman Kodak) or synthesized in our (G.S.) laboratory. Chemical structures and molecular weights (171 to 687) are shown in Fig 1. All compounds have in common a six-membered ring and methyl substituted alpha-carbons surrounding a nitroxyl group. Substitutions of the basic piperidinyl ring included alcohol, amide, aliphatic, ether and carboxylic acid groups. For each compound 10, 1, 0.1 and 0.01 mM solutions were prepared in normal human plasma (PL) and in deionized distilled water (AQ). The nitroxyl group has been shown to be stable over a wide range of pH using electron spin resonance (ESR).[9] For comparison purposes, plasma and aqueous solutions of gadolinium-DTPA (Schering AG) were prepared in identical concentrations. Samples of unadulterated deionized water and normal plasma served as reference standards to determine diamagnetic contributions to relaxation rates of solutions.

NMR spectroscopy

The proton spin-lattice relaxation times, T_1, were measured for all 10 mM samples in both aqueous and plasma solutions using 100 MHz (Varian XL 100) and 10.7 MHz (Praxis II) NMR spectrometers. The remaining concentrations (1, 0.1, and 0.01 mmol) of aqueous solutions were analyzed for T_1 on the 10.7 MHz spectrometer. A partial saturation recovery technique was employed to measure T_1 on the 10.7 MHz spectrometer. T_1 relaxation times on the 100 MHz spectrometer were determined by the inversion recovery method. Both 10.7 and 100 MHz measurements were performed at 23°C ± 1.5°C. The diamagnetic contributions to relaxation rate (as determined for

water and plasma standards) were subtracted from observed rates of NSL solutions to obtain the paramagnetic (p) contributions to T_1.[29]

ESR spectroscopy

Electron spin resonance (ESR) spectroscopy was performed on a Varian E-104A spectrometer operating at a 9.51 GHz resonant frequency. Solutions were contained within a 0.04 ml flat cell which was placed into the microwave cavity. The modulation amplitude of the magnetic field sweep was 20 gauss at 100 KHz, and a microwave power of 20 mW was used to record the first derivative of the absorption mode. The rotational correlation time (τ_r) of the NSL was estimated from the peak-to-peak linewidth in gauss of the low-field line on the first derivative absorption spectrum, using a conventional formula.[39]

Magnetic resonance imaging

All solutions placed in test tubes were imaged on a 3.5 kG spin-echo MRI system, operating at a hydrogen resonant frequency of 15 MHz.[18] The effective aperture for imaging was 6.5 cm. The imaging mode chosen yielded pixel sizes of 0.5 × 0.5 mm (XY) on a 128 × 128 matrix, and five contiguous transverse sections, each 4.4 mm thick, were sampled. Four separate spin-echo intensity images were obtained for each section, with each image containing echo delay times (TE) of 28 or 56 msec. Pulse repetition times (TR) of 0.5 or 2.0 sec were utilized. Spin-echo signal intensity values, in arbitrary units of voltage, were calculated for selected regions of interest.

Correlation times

An important variable influencing the degree of proton relaxation enhancement is the dipolar electron-nuclear correlation time, which describes the interaction time modulating translational diffusion of the NSL and water proton (τ_d), or the lifetime of a labile electron-nuclear complex (τ_c). The dipolar translational paramagnetic correlation time, τ_d, was estimated using the original theory developed by Hubbard.[27] (See Appendix). τ_d, the diffusion constant of solvent water protons (D), and distance of closest approach of protons to the paramagnetic center (d) all influence the contribution of "outer sphere" (long-range diffusion-mediated) relaxation (R_o) to spin-lattice relaxation. The paramagnetic correlation time, τ_c, was estimated by comparison of proton T_1 relaxation times at 100 MHz versus the 10.7 MHz frequency,[29] using conventional Solomon-Bloembergen (S-B) theory,[5,43] which assumes formation of a labile electron-nuclear complex via dipolar coupling (see Appendix).
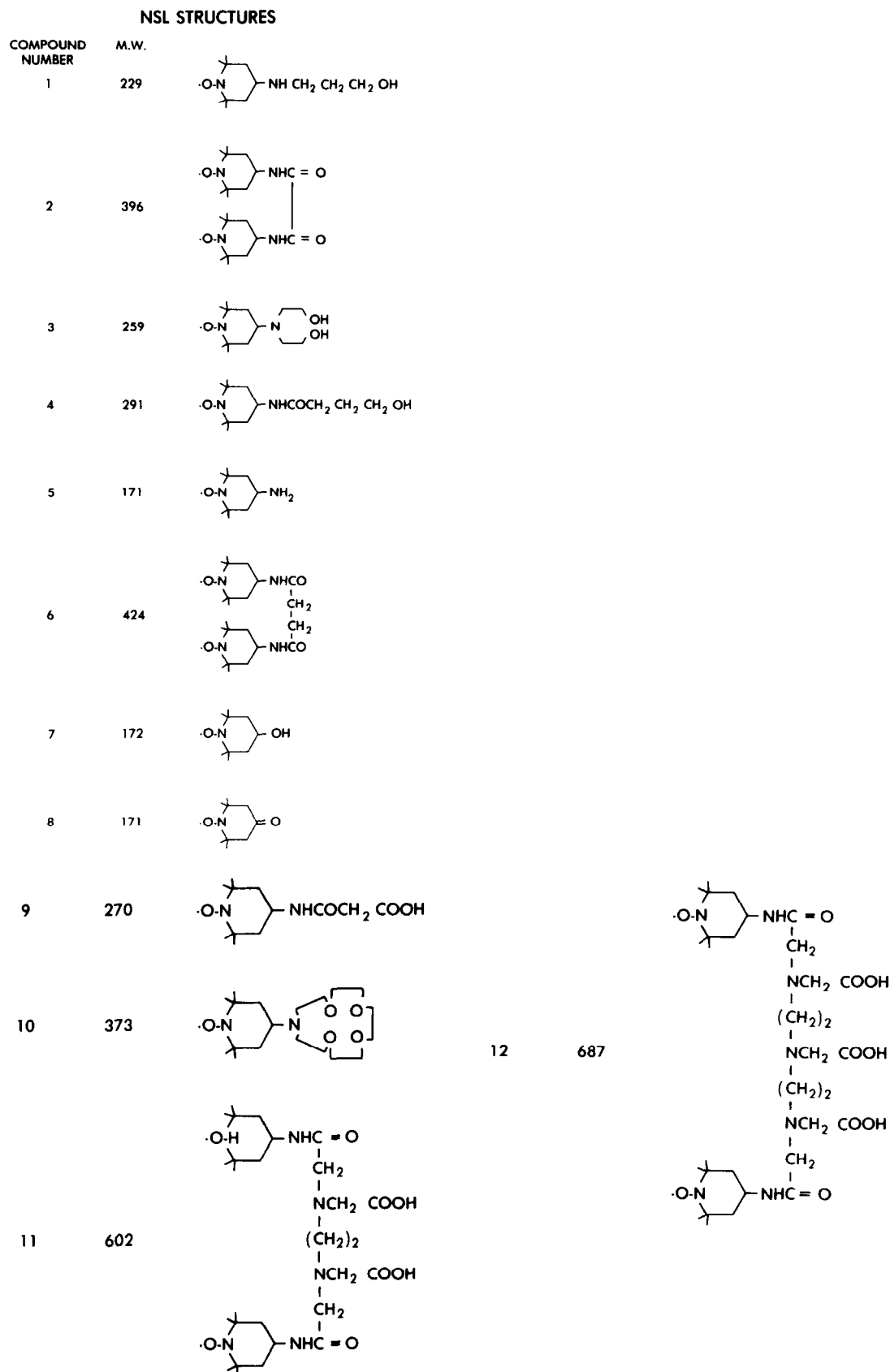
NSL STRUCTURES

COMPOUND NUMBER	M.W.	
1	229	·O-N⟩—NH CH$_2$ CH$_2$ CH$_2$ OH
2	396	·O-N⟩—NHC = O / ·O-N⟩—NHC = O
3	259	·O-N⟩—N⟨OH OH
4	291	·O-N⟩—NHCOCH$_2$ CH$_2$ CH$_2$ OH
5	171	·O-N⟩—NH$_2$
6	424	·O-N⟩—NHCO CH$_2$ CH$_2$ ·O-N⟩—NHCO
7	172	·O-N⟩—OH
8	171	·O-N⟩=O
9	270	·O-N⟩—NHCOCH$_2$ COOH
10	373	·O-N⟩—N⟨O O / O O⟩
11	602	·O-H⟩—NHC = O / CH$_2$ / NCH$_2$ COOH / (CH$_2$)$_2$ / NCH$_2$ COOH / CH$_2$ / ·O-N⟩—NHC = O
12	687	·O-N⟩—NHC = O / CH$_2$ / NCH$_2$ COOH / (CH$_2$)$_2$ / NCH$_2$ COOH / (CH$_2$)$_2$ / NCH$_2$ COOH / CH$_2$ / ·O-N⟩—NHC = O

Fig. 1. Chemical structures of nitroxyl spin labels studied in this investigation.

Table 1. Low field $1/T_{1_p}$ relaxation rates (sec^{-1}) for 10 mM solutions

Compound Number	10.7 MHz $1/T_{1_p}$(AQ)	10.7 MHz $1/T_{1_p}$(PL)
1	4.550	5.155
2	4.686	8.264
3	3.959	4.255
4	4.948	7.937
5	5.467	6.25
6	3.163	8.130
7	4.873	5.465
8	2.516	4.049
9	5.297	2.513
10	3.805	4.854
11	7.092	15.15
12	7.40	9.804
13*	75.19	69.93

*Gd-DTPA

RESULTS

Stability of NSL solutions

The NSL compounds were stable in aqueous and plasma solutions as measured by reproducible T_1 relaxation time measurements (less than 10% variation) over a period of three weeks.

Paramagnetic contribution to T_1 relaxation

Paramagnetic contributions to proton relaxation rates $(1/T_{1_p})$ were tabulated (Tables 1 and 2) to disclose trends among the various NSL's tested. Increasing proton relaxation rates were obvious with increasing concentration of paramagnetic NSL. Overall, $1/T_{1_p}$ increased to approximately 5 sec^{-1} for 10 mmol concentrations of paramagnetic nitroxyls in aqueous solution. For the 1 mmol aqueous solutions, $1/T_{1_p}$ increased to approximately 0.5 sec^{-1}. The $1/T_1$ of the deionized diamagnetic water reference standard was 0.4 sec^{-1}. No measurable increase in relaxation rates was seen with lower (.1 and .01 mmol) concentrations of nitroxyls.

$1/T_{1_p}$ relaxation rate enhancement was noticeably more effective at 10.7 MHz than at 100 MHz for both

plasma and aqueous solutions, and more effective in plasma compared to aqueous solutions for equimolar concentrations of NSL (see T_{1_p}(100MHz)/T_{1_p}(10.7 MHz) in Table 2). In water, a mean 1.70 fold (SD + 0.17) increase in $1/T_{1_p}$ relaxation rates was seen comparing low-field to high-field; in plasma, the mean increase was even higher (1.96, ±SD 0.14). The mean increases in $1/T_{1_p}$ relaxation rate enhancement in plasma versus equimolar aqueous solutions of NSL was 1.42 (±S.D. 0.53).

Relaxivity improved with ascending molecular weight. For example, NSL 1 (molecular weight 229) had $1/T_{1_p}$ relaxation rates much lower than NSL 11 (molecular weight 602) or NSL 12 (molecular weight 687) for both aqueous and plasma 10 mM solutions. The $1/T_{1_p}$ aqueous relaxation rate for NSL 1 was 4.55 sec^{-1} while NSL 11 was 7.09 sec^{-1} and NSL 12 was 7.40 sec^{-1}.

Perhaps a more important variable than molecular weight is the number of unpaired electron spins per molecule. Di-nitroxyls as opposed to mono-nitroxyls exhibited improved relaxivity, probably because the former have double the number of unpaired electrons capable of providing more proton relaxation enhancement. For example, NSL's 2, 11, and 12 with $1/T_{1_p}$ relaxation rates of 4.69 sec^{-1}, 7.09 sec^{-1}, and 7.40 sec^{-1} were more efficient than chemically similar mono-nitroxyls such as NSL 1 $(1/T_{1_p} = 4.55$ sec$^{-1})$.

Paramagnetic correlation times: Plasma versus aqueous solutions

To understand how the various NSLs might function in vivo as proton relaxation enhancers, all the NSLs in 10 mM concentrations were tested in plasma. In particular, it was helpful to determine if there were any differences in the estimated paramagnetic correlation time (τ_d or τ_c) of the NSLs between plasma and aqueous solutions. The pH on all plasma samples tested was between 7 and 7.5.

Using the translational diffusion model of Hubbard,[27] (see Appendix) we estimate a range of aqueous τ_d for the NSLs of $10^{-10.5}$ to 10^{-11} sec (see Table 2), in

Table 2. High Field $1/T_{1_p}$ relaxation rates (sec^{-1}) and correlation times (sec)*

Compound	100 MHz $1/T_{1_p}$(AQ)	100 mHz $1/T_{1_p}$(PL)	Aqueous $\dfrac{T_{1_p}(100\text{MHz})}{T_{1_p}(10.7\text{MHz})}$	Estimated Aqueous $\tau_d(-\log,\text{sec})$	Plasma $\dfrac{T_{1_p}(100\text{MHz})}{1/T_{1_p}(10.7\text{MHZ})}$	Estimated Plasma $\tau_d(-\log,\text{sec})$
1	2.288	2.632	1.65	10.7	1.82	10.5
2	2.826	2.336	1.66	10.7	3.54	10.0
3	2.288	2.288	1.73	10.8	1.86	10.5
?	2.112	2.865	1.50	10.5	2.84	10.2
11	3.600	5.155	1.97	11.0	2.93	10.2

*10 mM NSL concentration.

general agreement with previous reports.[21,23] These estimations of the diffusional correlational time were made by comparing T_{1_p}'s at 100 MHz versus 10.7 MHz, assuming that the electron-nuclear dipolar interaction modulating water proton relaxation is described by the relative translational diffusion of the water proton and the NSL electron.

Aqueous τ_c was estimated by the ratio of T_{1_p} relaxation at 100 MHz versus 10.7 MHz,[29] using the standard Solomon electron-nuclear dipolar equations,[43] ignoring the scalar term of Bloembergen.[5] For all aqueous solutions of nitroxyls, τ_c was estimated to be between $10^{-10.6}$ and $10^{-10.3}$ seconds. This estimate was based on the published value of aqueous τ_r (rotational correlation time) for NSL 5 (MW 171, $\log \tau_r = -10.4$ sec at 20°C)[39] and for NSL 8 (MW 171, $\log \tau_r - 10.9$ sec at 20°C)[37], as well as our own ESR spectral calculations of τ_r on these NSL's. We assumed that τ_r, not τ_m, was the rate limiting correlation time for the Solomon-Bloembergen model of NSL-relaxation enhancement. These τ_r values led us to choose the longer of two possible τ_c values at each value of the ratio of T_{1_p} (100 Mhz)/T_{1_p} (10.7 MHz) for the NSLs. Without these τ_r values, an unambiguous determination of aqueous τ_c would not be possible.

Applying the Hubbard diffusion-model to the plasma NSL solutions, we estimate τ_d to range from 10^{-10} to $10^{-10.5}$ sec. This is in reasonable agreement with our calculation of τ_d from the NSL-protein dispersion studies of Polnaszek and Bryant ($10^{-9.9}$ sec),[41] which was performed over a wide range (0.01MHz to 30MHz) of numerous different proton resonant frequencies on a field-cycling spectrometer. Thus, we would conclude that the microviscosity of water protons in plasma diffusing near the nitroxyl paramagnetic center is higher (slower diffusion) than in pure water, a conclusion supported by pulsed-gradient spin-echo NMR translational self-diffusion measurements of Cooper, et al.[16] In that study, the diffusivity ratio of bulk water in human plasma versus pure water was found to be 0.86. Polnaszek and Bryant[41] found much slower water self-diffusion at the surface (defined as the first several monolayers of water) of their spin-labelled protein. Their diffusivity ratio of surface water on the spin-labelled protein relative to an aqueous spin label solution was 0.19.

In plasma, at least three nitroxyls emerged as having much longer estimated τ_c's, down to $10^{-8.5}$ to $10^{-8.6}$ sec (NSL 2, 6, 11). These estimations of exchange correlation times were measured by comparing nitroxyl paramagnetic T_{1_p}'s at 100 MHz versus 10.7 MHz.[29] The data on these three nitroxyls show that T_1 decreased to an extent that the paramagnetic relaxation rate enhancement ratio (E) roughly doubled at 10.7 MHz

(Table 1), comparing the ratio of 10 mM $1/T_{1_p}$ in plasma NSLs to 10 mmol $1/T_{1_p}$ aqueous NSLs. We were only able to assign a single, unambiguous τ_c to a NSL in plasma if the ratio of T_{1_p} (100 MHz)/T_{1_p} (10.7 MHz) exceeded a minimum value of 2.6. This value (2.6) was obtained from the peak T_{1_p} ratio calculated from the Solomon equation for a range of τ_c's (10^{-12} to 10^{-10} sec). For the nine NSLs where high field and low field 10 mM plasma T_{1_p}'s were measured, the mean ratio was 1.96, SD ± 0.14, range 1.82 to 2.18. For T_{1_p} (100 MHz)/T_{1_p} (10.7 MHz) ratios of less than 2.6, three different τ_c values are possible.

Electron spin resonance spectroscopy of these three NSL's (2, 6, 11), plus two other NSL's (4, 5) in water and plasma failed to demonstrate any lengthening of τ_r from water to plasma. ESR spectra are very sensitive indicators of the rotational molecular motion of the NSL.[4] The absence of any lengthening of τ_r from water to plasma for all NSL studied by ESR is a strong argument against the use of the Solomon-Bloembergen exchange model for NSL-induced water proton relaxation enhancement, especially in plasma. Since τ_r (determined by ESR) for the NSL in plasma was the same as in water ($\tau_r = 10^{-10.4}$ to $10^{-10.9}$ sec), τ_c in plasma can be no longer than $10^{-10.4}$ sec. Thus, a model which calculates τ_c for three NSL at $10^{-8.5}$ to $10^{-8.6}$ sec must be considered inadequate. Our data thus supports the consensus in the literature[20,22,24,27,28,35,40,41] that NSL-induced solvent proton relaxation is predominately modulated by the relative translational self-diffusion of water protons near the NSL's unpaired electron.

MRI signal intensity

Signal intensity data observed for the NSL solutions showed that maximal relaxation enhancement did not correspond with maximal MRI signal intensity (Table 3). Because spin-echo MRI depends on both T_1 and T_2 relaxation times, T_1 shortening generally acts to increase image intensity; however, T_2 shortening also observed with paramagnetics usually acts to decrease signal intensity.[46] The observed MRI signal intensity is highly dependent on the TR and TE values chosen. A paramagnetic that preferentially shortens T_1 over T_2 will have the highest signal intensity, but could conceivably be considered a relatively inefficient proton relaxation enhancer. In particular, at pulse repetition times of 500 ms, NSL 11 was found to have the highest MRI signal intensity. In fact, several nitroxyls had greater signal intensity at 10 mM concentrations than gadolinium-DTPA—another MRI contrast agent.[45,47] This occurred because gadolinium-DTPA's relaxation enhancement dominates at this concentration, producing decreased spin-echo signal intensity.

Magnetic Resonance Imaging ● Volume 3, Number 1, 1985

Table 3. MRI signal intensities

Compound	MRI Intensity[a] (TR = 500 msec, TE = 28 msec)		Rank order of intensities (TR = 500 msec)	
	10 mM Conc.	1 mM Conc.	10 mM	1 mM
1	11056	5908		
2	5902	3816	11	6
3	4796	4423	10	9
4	5642	3518	12	5
5	14378	6177	9	1
6	11988	7183	5	12
7	11128	5216	6	10
8	11722	5210	8	11
9	14561	6335	7	7
10	19928	5852	1	8
11	19998	5544	2	3
12	19690	5893	4	2
			3	4

[a]in arbitrary units of mean pixel voltage

DISCUSSION

The $1/T_{1_p}$ relaxation rate enhancement observed from NSLs was noticeably more effective at 10.7 MHz than 100 MHz, and in plasma compared to water. Our data does not permit a complete description of the magnetic field dependence for NSL-induced solvent $1/T_{1_p}$ relaxation rate enhancement. Precise characterization of solvent water outer sphere diffusion contributions to T_{1_p} would be best accomplished using field-cycling spectrometer measurements of T_{1_p} relaxivity at multiple magnetic field strengths ("dispersion" studies) and temperatures, with subsequent quantitative analysis in light of various theories of outer sphere relaxation.[24,27,28,40] The clinically relevant result reported herein is that NSL-induced T_1 water proton-relaxation rate enhancement is greater at lower MRI field strengths, with more dispersion between fields than reported for other potential MRI contrast agents including Mn-DTPA and gadolinium-DTPA.[31] Our in vitro measurements of the T_{1_p} relaxivity of aqueous Gd-DTPA at 10.7 MHz and 100 MHz showed near-identical T_{1_p}'s.

NSL's are not new to biophysical research and have been used for two decades as "spin-labels" for in vitro biological studies.[44] Brasch and others have reported on a water soluble piperidinyl NSL derivative which is rapidly excreted in the urine after intravenous injection and provides physiological data on renal excretion by MRI urography.[8-10] These investigators further point out that NSL's could serve to differentiate iso-intense abnormal tissues (e.g. tumor) from adjacent normal tissues. Paramagnetic contrast agents have potential to aid in assessments of capillary perfusion and organ function by paramagnetic labeling of cells and tissue-specific biomolecules.[10,44] In addition,

NSLs can help to assess the integrity of the blood-brain barrier.[10] NSLs have also been used in the in vitro study of drug metabolism by covalent binding to drugs such as steroids.[3]

Toxicity tests of two prototype NSL contrast agents for possible mutagenesis and carcinogenesis have been performed. A modern and sensitive assay for chemical carcinogens is sister-chromatid exchange testing.[50] Using this method, no evidence of mutagenesis for nitroxyls or their reduced metabolites was found.[1] Furthermore the LD_{50} in rats (>15 mmol/kg) has been demonstrated to be greater than 100 times the lowest effective dose for renal enhancement on spin echo images (0.14 mmole/kg).

The NSLs tested were not prone to spontaneous in vitro reduction. The measurements of proton relaxation times in water and plasma were reproducible over a period of three weeks on both the MR imager and the MRI spectrometer. The nitroxyl moiety itself is relatively stable. For example, it remains unchanged when heated up to 123°C and over a pH range of 1.7–10. It can be stored at room temperature as a solid for 60 days or longer without loss of activity as documented by ESR spectroscopy.[9] Yet, studies have shown that nitroxyls are quire susceptible to partial in vivo chemical reduction, perhaps from ascorbic acid or enzymes.[17,26] Despite in vivo bioreduction, effective MRI contrast enhancement occurs.[8-10]

In plasma, NSL demonstrated definite lengthening of the dipolar diffusional correlation time (τ_d) in association with improved proton relaxation enhancement. This outer sphere diffusional paramagnetic correlation time, τ_d, was lengthened from water to plasma in all the NSL's. As noted in the Appendix, the preponderance of previous work in the literature with NSL-solvent relaxation rate enhancement would favor an outer sphere solvent diffusion mechanism for the nitroxyl-water interaction.[11,20-24,27,28,35,40,41]

The importance of the NSL paramagnetic correlation, τ_d, in effecting proton relaxation enhancement is not only suggested by our in vitro studies in plasma, but can also be inferred from previous in vivo MRI studies with NSL's. Brasch et al.[10] found dramatic T_1 shortening induced by NSL 9 on a 15 MHz MRI system in vivo for a canine cerebral abscess despite local ESR-determined NSL tissue concentrations of only 0.45 mM. In water, a 1 mM concentration of NSL 9 shows very little T_1 shortening (T_{1_p} equals 1666 ms, T_1 of water equals 2500 ms). Thus, we may conjecture that in the tissue milieu in vivo, long paramagnetic correlation times, presumably resulting from NSL-water-macromolecular interactions, can enhance proton relaxation much greater than in a pure aqueous milieu. For instance, the rotational correlational time (τ_r) of a low molecular weight NSL bound to bovine

brain hexokinase has been measured by ESR at a value of 4.2 × 10^{-9} sec.[38] However, water translational diffusion is more important than the rotational tumbling rate of the NSL in determining NSL relaxivity.[41] The microviscosity of water in biological tissues, as measured by the translational molecular self-diffusion coefficient, is at least 50% less than free water.[16] Our studies would also lead to a prediction that in vivo fluid or tissue PRE on a 100 MHz MRI system could be less dramatic than on a 10.7 MHz system, for equivalent concentrations of NSL's, depending on the degree of diamagnetic T_1 lengthening in the tissue of interest at the higher field.

CONCLUSION

In summary, T_1 relaxation-rate enhancement of solvent water protons by nitroxyl spin labels is dependent on the static magnetic field strength. NSLs that have large molecular weights and interact with slower-diffusing water molecules near plasma macromolecules cause greater proton relaxation rates than equimolar concentrations of small molecular weight aqueous NSL's. This is due to slowing of the correlation time, from slower solvent water translational self-diffusion. In addition, dinitroxyls without ESR evidence of strong electron spin-spin interaction had better relaxation enhancement. Unlike certain other paramagnetic metal or rare earth complexes, nitroxyl spin-labels can have variously substituted configurations to influence their biodistribution and thereby enhance their clinical utility.

APPENDIX

In a solution of NSL in water or in plasma, the nuclear spin of solvent water protons will be relaxed by one of two possible interactions with the unpaired electron on the NSL. The first interaction is formation of a labile molecular complex between the nuclear spin and electron spin via electron-nuclear dipolar coupling (Term A below) or scalar (contact interactions (Term B below). The effectiveness of paramagnetic relaxation enhancement will then depend on many variables including the magnetic moment, electron-nuclear distance, field strength, and paramagnetic correlation time, τ_c. Relaxation rates $(1/T_{1M})$ of a proton in the vicinity of an unpaired electron are described by the Solomon-Bloembergen[43,5] equation outlined below, which assume formation of a transient electron-nuclear complex:

$$\frac{1}{T_{1M}} = \frac{2}{15} \frac{S(S + 1)\gamma^2 g^2 \beta^2}{r^6} \left(\frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} + \frac{7\tau_c}{1 + \omega_s^2 \tau_c^2} \right)$$
$$+ \frac{2}{3} \frac{S(S + 1)A^2}{\hbar^2} \left(\frac{\tau_e}{1 + \omega_s^2 \tau_e^2} \right)$$

where S is the electron spin quantum number; g is the electronic g factor; β is the Bohr magneton; ω_I and ω_s ($= 657\, \omega_I$) are the Larmor angular precession frequencies for the nuclear spins and electron spins, respectively; r is the ion-nucleus distance; A is the hyperfine coupling constant; and τ_c and τ_e are the correlation times for the dipolar and scalar interactions, respectively, where $1/\tau_c = 1/\tau_r + 1/T_s + 1/\tau_m$. τ_r is the rotational correlation time of the vector between the unpaired electron and the nuclear spin,[36] T_s is the electron spin relaxation time, and τ_m is the lifetime of the nuclear spin in the sphere of influence of the paramagnetic center. Based on the relaxation data and conclusions reached by Endo et al.,[23] NSL-induced solvent proton relaxation is governed predominately by dipolar coupling (Term A), and scalar contributions (Term B) can be ignored.

If τ_c equals the reciprocal of the nuclear precessional frequency, T_1 relaxation rate enhancement is maximal (i.e. $1/\tau_c$ increases with more optimal τ_c's).[29] To increase τ_c, one must know its limiting variable. Nitroxyls possess naturally long electron spin relaxation times (spin lattice relaxation time, $T_{1_e} = 10^{-5}$ sec; electron spin-spin relaxation time, $T_{2_e} = 10^{-8}$ sec) which are at least 2 orders of magnitude longer than any other paramagnetic metal or lanthanide ion.[21] The literature[37,39] and our ESR measurements have calculated a τ_r of approximately 10^{-11} seconds for the low molecular weight NSLs currently undergoing evaluation as prototype MRI contrast agents. Thus, either τ_r or τ_m could be a limiting variable in determining τ_c for NSLs. Because T_s is long at 10^{-8} sec, one could theoretically manipulate τ_r or τ_m. To lengthen nitroxyls' τ_r beyond 10^{-11} seconds, and since T_s can only get longer at higher magnetic fields, one must increase their molecular weight and/or bind NSLs to macromolecules. This will slow the rate at which nitroxyls tumble within solution. This may increase the proton relaxation rate, if τ_m is not the limiting variable (assume $\tau_m > 10^{-11}$ seconds). Thus, proton relaxation enhancement could conceivably improve as τ_c approaches the reciprocal of the nuclear processional frequency, if one accepts the fundamental Solomon-Bloembergen assumption of formation of a labile electron-nuclear dipolar complex. Endo et al.[23] point out that their investigation of NSL-solvent relaxation-dispersion supported the application of Solomon-Bloembergen theory when proton-donor solvents were used. Certainly, water is a proton donor but they did not specifically study water as a solvent for NSL T_1 relaxation-enhancement. In theory, the ability to form such a labile electron-nuclear complex may be hampered by the common structural feature of NSLs, where four bulky methyl groups sterically hinder access of water to the unpaired electron on the nitroxyl

moiety. More importantly, experimental data[20,22,41] has indicated the inadequacy of the Solomon-Bloembergen model in describing NSL-induced solvent relaxation.

Thus, a second approach to understanding solvent proton-NSL electron interactions has evolved. These theories of Hubbard[27] and Freed[24,28] (based on earlier principles of outer sphere relaxation established by Bloembergen, Purcell, and Pound,[6] and Pfeiffer[40]) assume that *no* chemical interaction between these two spins occurs. Rather, the electron-nculear dipolar interaction modulating water proton relaxation is described by the relative *translational diffusion* of the water proton and the NSL electron. The outer sphere relaxation process is characteristically seen with inaccessible "buried" paramagnetic centers, where a long range magnetic interaction between diffusing solvent water protons and the paramagnetic center induces fluctuations in the local magnetic field seen by the proton.[32,33] One could regard the paramagnetic center of nitroxyls as being "buried", both by the sterically-hindering bulky methyl moieties on the organic ring, as well as by delocalization of the unpaired electron onto the nitrogen atom, making outer sphere effects important. Since the translational molecular self-diffusion coefficient of water is much greater than that of the NSL, the proton relaxation-enhancement induced by NSL is felt to be predominately dependent on water translational motion.[11] In fact, Polnaszek and Bryant[41] have measured the translational self-diffusion coefficient of water on the surface of a nitroxyl-protein, applying the force free diffusion model of Freed[24,28] to T_1 measurements of NSL-protein complex in solution over a wide range of magnetic fields. Thus, for MRI, the local microviscosity of water in the various biological tissues and fluids may well determine the magnetic field dependence of NSL-induced proton-relaxation enhancement.[11] Now that we have developed a method for water self-diffusion measurements in MRI,[48,49] this theory could eventually be tested *in vivo*.

Scalar coupling of free radical electrons with solvent nuclei is important with nuclei other than protons, such as ^{13}C, ^{19}F, and ^{31}P.[20] With these nuclei, both diffusion models and "sticking" models have been used to describe the scalar interaction.

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