In vitro Characterization of MS-325 by Multinuclear Relaxometry

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Introduction

This work reports the *in vitro* physicochemical characterization of MS-325, a new blood pool agent for magnetic resonance imaging[1-3]. Proton relaxivity (r_1) in water at various magnetic fields and temperatures, exchange time of the coordinated water molecule (τ_M), binding to human serum proteins as well as kinetic and thermodynamic stabilities were studied.

Materials and Methods

MS-325 was synthesized as described by McMurry et al. [4,5].

The exchange time of the coordinated water molecule, τ_M, was obtained from the analysis of the temperature dependence of the oxygen-17 transverse relaxation rate in aqueous solution (Bruker AMX-300 spectrometer, 7.05 T, proton decoupled spectra)[6].

Proton longitudinal relaxation dispersion profiles were measured at 5, 15, 25, 37 and 45°C over a field range extending from 0.02 to 300 MHz (Field Cycling Relaxometer, FCS, Honesdale, NJ, and Bruker AMX-300 spectrometer).

Interactions with proteins were studied by proton relaxometry at 37°C with non defatted Human Serum Albumin (HSA 4% w/v, Sigma) and serum (Kontrollogen L, Boehringer).

Kinetic and thermodynamic stabilities were studied at 37°C through the evolution of the P-31 longitudinal relaxation rate (R_1) of ATP in solutions containing MS-325 (Bruker MSL-200 spectrometer)[7].

Transmetallation by Zn(II) ions was evaluated by the decrease of the water proton longitudinal relaxation rate at 37°C and 20 MHz (Bruker Multispec PC 20) of buffered solutions (pH 7, phosphate buffer) containing 2.5 mM of the gadolinium complex and 2.5 mM of Zn(II).

Results and Discussion

The τ_M value (83±13 ns at 37°C) obtained by O-17 NMR clearly indicates that the water exchange does not limit proton relaxivity. This is confirmed by the evolution of the proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles versus temperature (fig. 1). It is worth mentioning that, in water at 37°C and 20 MHz, the proton relaxivity of MS-325 is roughly 1.4 times larger than that of Gd-DTPA.

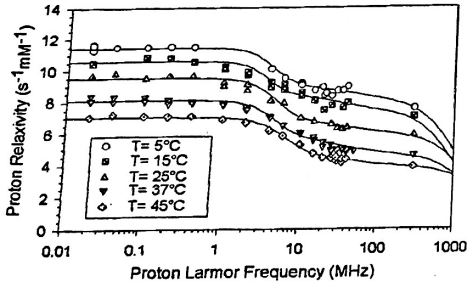

Figure 1: Proton NMRD profiles of MS-325 at various temperatures

The P-31 NMR study of the stability shows that as for the parent compound, Gd-DTPA, a competition takes place between ATP and MS-325 for the gadolinium ion. However: i) the equilibrium is reached much more slowly (24 hrs) than for Gd-DTPA (1 hr), and ii) the steady state values of P-31 R_1 of ATP are lower than for Gd-DTPA. Both kinetic and thermodynamic stabilities of MS-325 are therefore higher than for Gd-DTPA. Similarly, the slower and rather limited evolution of water paramagnetic longitudinal relaxation rate (R_1^p) in aqueous solution containing phosphate buffer and Zn(II) agrees with a high stability of MS-325 towards transmetallation by Zn(II) (fig. 2).

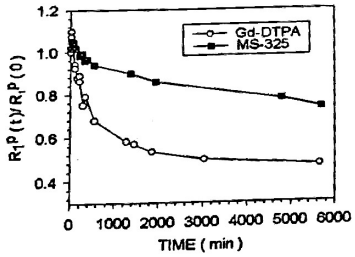

Figure 2: Time evolution of the water proton paramagnetic relaxation rate relative to its initial value (T= 37°C).

The interaction of MS-325 with serum proteins and with HSA is clearly demonstrated by the proton NMRD profiles (fig. 3).

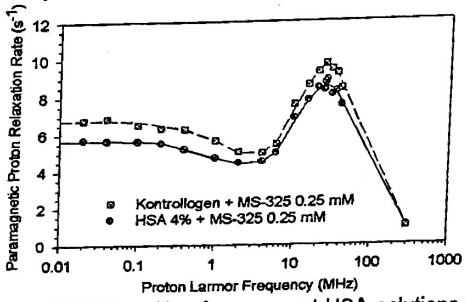

Figure 3: Proton NMRD profile of serum and HSA solutions containing 0.25 mM of MS-325 (T = 37°C).

Assuming one binding site of high affinity on HSA, a value of 6100 ± 2130 M^{-1} was calculated for the association constant from the evolution of the proton paramagnetic relaxation rate of solutions containing 4% of HSA and various concentrations of MS-325 (0.0625 mM to 2.08 mM) at 0.47 T and 37°C. Under these conditions, the paramagnetic relaxation rate induced by 1 mM of MS-325 in a 4 % HSA solution is 4.6 times larger than in pure water solution; this corresponds to an *apparent relaxivity* of approximately 25 $s^{-1}mM^{-1}$. The apparent relaxivity, however, strongly depends on the proportion of MS-325 bound to HSA and is close to 40 $s^{-1}mM^{-1}$ (ν_0 = 20 MHz) when [MS-325]=0.0625 mM and [HSA]= 4%. Using the observed paramagnetic relaxation rate in HSA 4% at various magnetic fields (fig. 3) and the calculated association constant, the relaxivity profile of MS-325 bound to HSA was estimated. Its maximum (44-46 $s^{-1}mM^{-1}$) is reached between 20 and 35 MHz. The fitting of the profile indicates that the mobility of the paramagnetic center (τ_R = 2.4-2.8 ns) is higher than expected for a complex fully immobilized on the surface of the protein.

References

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