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Short Communication

The presence of halide salts influences the non-covalent interaction of MRI contrast agents and human serum albumin

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ABSTRACT: The rationale and objectives of the study were to evaluate the influence of the experimental conditions (buffer, salt, etc.) on the data characterizing the non-covalent interaction between MRI contrast agents and human serum albumin and hence their *in vivo* relaxivity. The interaction of three gadolinium contrast agents (Gd-EOB-DTPA, Gd-BOPTA and MP-2269) with human serum albumin was assessed through the measurement of proton relaxation rate enhancement in various experimental conditions. The data show the negative effect of halide salts on the paramagnetic relaxation enhancement of the three contrast agents. The presence of halide salts can thus have a negative effect on the efficacy of MRI contrast agents interacting with HSA. In addition, careful attention must be paid to comparisons of the binding parameters of various contrast agents reported in different studies since the composition of the medium can greatly influence the non-covalent interaction.

KEYWORDS: relaxometry; contrast agent; binding; salts

INTRODUCTION

Paramagnetic chelates of gadolinium, which modify the image contrast through their effects on the nuclear magnetic relaxation of the tissue water protons, are currently used in clinical MRI (1). The efficiency of a contrast agent is usually expressed as its relaxivity r_1, which is the enhancement of the water proton relaxation rate induced by 1 mM of the contrast agent. Several parameters influence the relaxivity, such as τ_R, the rotational correlation time, τ_M, the residence time of water molecules bound in the first coordination sphere of the metal complex, q, the number of these water molecules, and $\tau_{s1,2}$, the electron spin relaxation times (2).

In theory, the efficiency of a contrast agent can be improved by the increase of its rotational correlation time τ_R. One way to increase this parameter without modifying too much the other factors is the formation of non-covalent interactions between the paramagnetic complex and a macromolecule. Investigations have focused on human serum albumin (HSA), the most abundant protein in blood plasma, which transports numerous endogenous and exogenous compounds (3). To favour this kind of interaction, Gd-DTPA has been substituted by lipophilic molecules in order to increase its affinity for the protein. Such interactions result in a prolonged residence time of the complex in the vascular system, making it useful for angiography or improving its availability for molecular imaging. Gd-EOB-DTPA (4–6), Gd-BOPTA (7–9) and MP-2269 (10–13), a Gd(III) complex of 4-pentyl-bicyclo[2.2.2]octane-1-carboxyldi-L-aspartyllysine-derived DTPA, have been reported to bind to HSA.

The measurement of the binding constants is of paramount importance in this context, but some discrepancies between published studies (5,9,14,15) prompted us to assess the influence that experimental conditions may have on the affinity of these three contrast agents for HSA.

RESULTS AND DISCUSSION

Previous studies, based on the shape of nuclear magnetic dispersion resonance (NMRD) profiles, showed that the proton longitudinal relaxation rates of Gd-BOPTA (9), Gd-EOB-DTPA (5,6) and MP-2269 (10) in albumin solution were enhanced as compared with their values measured in water. A non-covalent interaction with HSA

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reducing the mobility of the Gd complex was responsible for this effect. A rigorous quantitative evaluation of this interaction was undertaken in the present work through the analysis of the proton relaxation rate enhancement. In this protocol, a constant concentration of HSA, close to its concentration in blood (4%), was used while the concentration of the paramagnetic complex varied from 0.1 to 2 mM. The fitting of the proton paramagnetic relaxation rates measured as a function of the concentration was then carried out according to eqn (1) (6). In water, the association constants (K) obtained (Fig. 1, Table 1) are in good agreement with those previously reported (5,9,13). For Gd-BOPTA and Gd-EOB-DTPA, which show similar but moderate affinities for HSA, one binding site is involved. MP-2269, on the contrary, has a stronger affinity for the protein and two binding sites. With this range of concentration ratio ([contrast agent]/[HSA] = 0–3.5), the number of sites was confirmed by electrospray mass spectrometry (16,17). In a buffer solution (10 mM Na_2HPO_4, 150 mM NaCl, pH 7.4), the relaxivity of the Gd complexes remains unchanged as compared with water solutions ($r_1 = 5.5\,s^{-1}\,mM^{-1}$ for Gd-EOB-DTPA, $4.7\,s^{-1}\,mM^{-1}$ for Gd-BOPTA, and $6.2\,s^{-1}\,mM^{-1}$ for MP-2269), but the paramagnetic relaxation enhancement in the presence of HSA is significantly smaller for all complexes as compared with the values obtained in the non-buffered water solution (Fig. 1). This relative attenuation is much more pronounced for Gd-EOB-DTPA and Gd-BOPTA than for MP-2269: for a 1 mM complex concentration, decreases of 42, 49 and 19% are observed for Gd-EOB-DTPA, Gd-BOPTA and MP-2269 respectively. This can be related to the highest affinity of MP-2269 for HSA and/or to different binding sites of the Gd-complexes. The fittings of the data show that all contrast agents have a weaker affinity for HSA than in water solution (Table 1).

The presence of phosphate, sodium ions and chlorine thus decreases the strength of the non-covalent interaction. The major effect of NaCl was assessed in solutions containing 1 mM Gd-EOB-DTPA, with and without 4% of HSA, and increasing amounts (from 0 to 600 mM) of the salt (Fig. 2). The paramagnetic relaxation rate of the contrast agent in protein-free solution is barely influenced by the salt concentration. At the very most, a slight increase can be observed at very high salt concentrations due to the slight decrease of water concentration and to the small viscosity increase of the solution. On the contrary, in protein-containing solutions (4% HSA, 1 mM Gd-complex), the relaxation rate drops markedly from about $\sim 14\,s^{-1}$ in the absence of salt to $\sim 9\,s^{-1}$ when [NaCl] = 150 mM and $\sim 8\,s^{-1}$ when [NaCl] = 600 mM. The principal role of the Cl^- anion was demonstrated by the use of other electrolytes, NH_4Cl, KCl, KF, KBr and KI. The observed decreases of the relaxation rates were indeed similar for all electrolytes containing chloride anion, independent of the nature of the cation. The various potassium salts had markedly different negative effects:

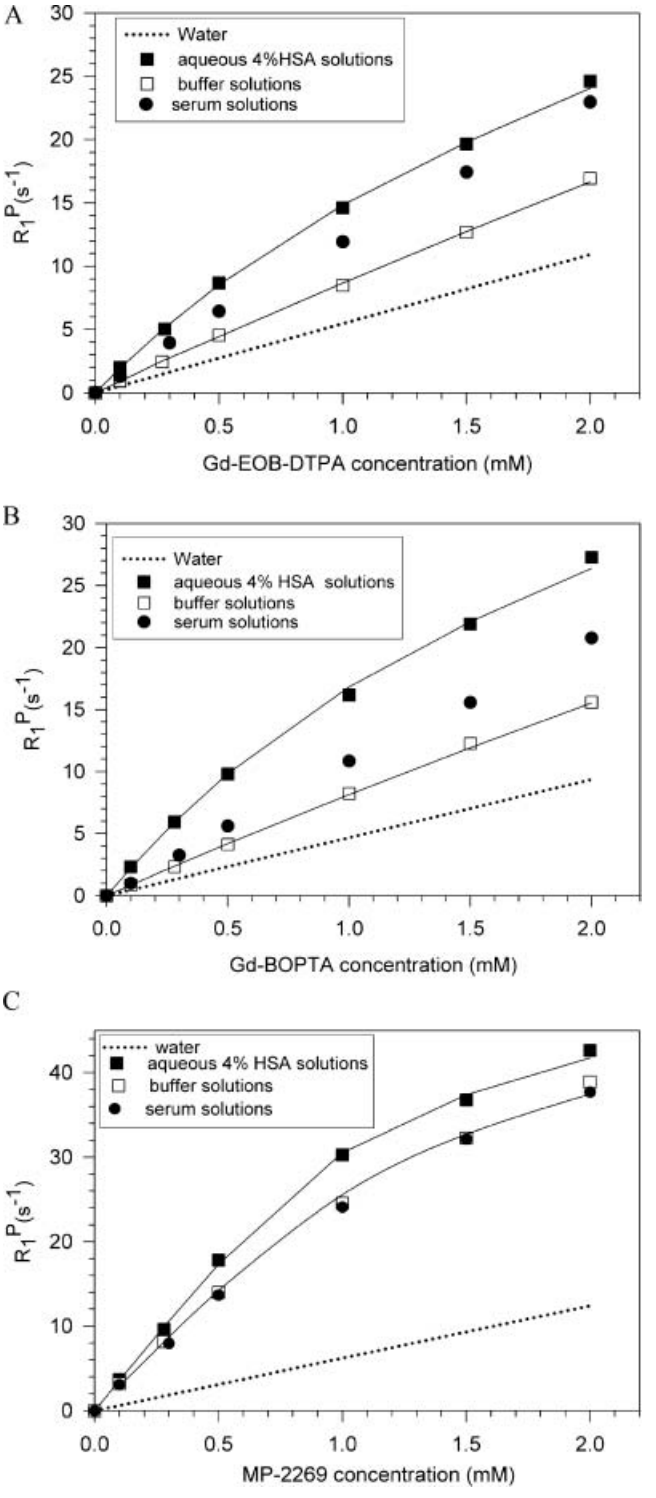

Figure 1. Proton paramagnetic relaxation rates R_1^p (s^{-1}) (20 MHz, 310 K) of aqueous 4% HSA solutions, 4% HSA in buffer solutions (10 mM Na_2HPO_4, 150 mM NaCl, pH 7.4) and serum solutions for increasing amounts of the gadolinium complex (A, Gd-EOB-DTPA; B, Gd-BOPTA; and C, MP-2269). The continuous lines correspond to the fitting of the data and the dashed lines represent R_1^p in a water solution free of albumin (experimental data points not shown).

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Table 1. Results of the fitting of the paramagnetic relaxation rates of 4% HSA solutions (Fig. 1) with eqn (1)

	K (10^3 M^{-1})	N	r_1^c (s^{-1} mM^{-1})	r_1^f (s^{-1} mM^{-1})
Gd-EOB-DTPA				
Water	1.5 ± 0.5	0.9 ± 0.3	37.3 ± 16.8	6.0 ± 1.0
Buffer solution	0.2 ± 0.01	1.0 ± 0.5	35.5 ± 0.5	6.6 ± 0.6
Gd-BOPTA				
Water	1.5 ± 0.5	1.0 ± 0.4	42.9 ± 11.1	5.2 ± 0.7
Buffer solution	0.2 ± 0.09	1.0 ± 0.2	37.5 ± 9.2	5.1 ± 0.5
MP-2269				
Water	16.0 ± 6.9	1.6 ± 0.1	38.0 ± 7.4	6.5 ± 0.3
Buffer solution	9.7 ± 3.6	1.8 ± 0.2	32.0 ± 2.5	6.5 ± 0.4

the larger the halide, the greater the effect (KI > KBr > KCl > KF; Fig. 2). Similar results were obtained with Gd-BOPTA, for which the R_1^p of a 1 mM solution of Gd-BOPTA in HSA 4% decreased by ~40% in the presence of NaCl and KCl 150 mM, and by 54% in presence of KI 150 mM.

These results agree with previously published data showing that the binding of halide anions increases when going from F^- to Cl^- to Br^- to I^- and that the Cl^- ions reduce the strength of the binding interaction of serum albumin with other ligands (18–21). How halide ions influence the binding of other ligands is still unclear but the size of the halide seems to play a role.

In order to make the experimental conditions closer to the *in vivo* situation, we also tested the behaviour of the three complexes in reconstituted serum (Kontrollogen L). Indeed, in addition to HSA, the serum contains lipids, substrates, electrolytes and other proteins that could influence the non-covalent bindings. We observed a global decrease in the paramagnetic relaxation rates of all complexes as compared with their values in 4% HSA solution in water (Fig. 1). This again can be related in part to the presence of chloride anions ([Cl^-] = 107–111 mM)

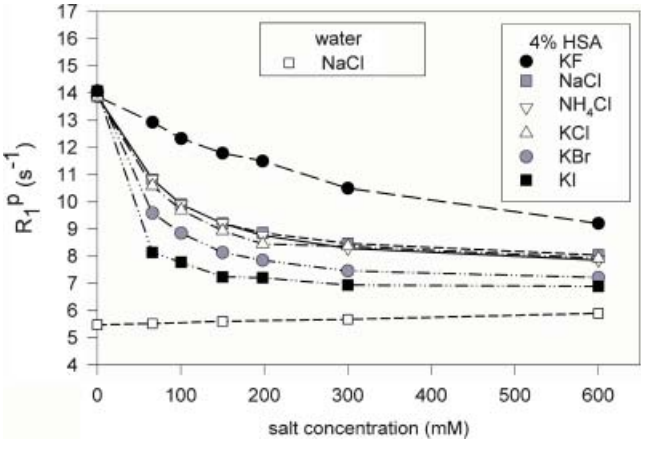

Figure 2. Evolution of the paramagnetic relaxation rates R_1^p (s^{-1}) (20 MHz, 310 K) of 1 mM Gd-EOB-DTPA (with and without 4% HSA) as a function of the nature and the concentration of the added salt.

and possibly to the presence of other components able to bind to HSA. The viscosity of serum Kontrollogen, which is 1.41 larger than that of a 4% HSA solution at 310 K, could explain the smaller relaxivity decrease observed for Gd-EOB-DTPA and Gd-BOPTA.

CONCLUSION

The present work shows that the interaction of the three gadolinium complexes studied with the HSA protein can significantly vary as a function of the composition of the medium. The data clearly show that the non-covalent interaction with HSA is strongly reduced in the presence of sodium chloride and other halide salts. In addition to the presence of fatty acids (22,23) and other experimental conditions like the temperature and the pH, the presence of halide anions has to be taken into account when studying the binding of gadolinium contrast agents to HSA. It has to be stressed, therefore, that a reliable comparison of the binding data is meaningful only if the experiments are performed in similar conditions. Predictors about the *in vivo* efficiency of albumin binding contrast agents are thus likely to be overestimated if they are based on studies performed in simple aqueous solutions.

MATERIAL AND METHODS

Non-defatted HSA A-1653 (Sigma, Bornem, Belgium), Gd-EOB-DTPA (Primovist®) (Schering AG, Berlin, Germany), Gd-BOPTA (Bracco SpA, Milan, Italy) and MP-2269 (Mallinckrodt, Saint-Louis, USA) were used without further purification. Kontrollogen was purchased from Hoechst-Behring (France).

The proton relaxation rates (R_{1obs}) were measured at a fixed field of 0.47 T and at 310 K using a Minispec-pc 20 (Bruker, Karlsruhe, Germany). The proton paramagnetic relaxation rates (R_1^p) were obtained by subtracting the diamagnetic contribution of the medium from R_{1obs}. The resulting data obtained in HSA solutions were fitted using

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eqn (1) (6):

$$R_1^{\mathrm{p}} = 1000\left[r_1^{\mathrm{f}}L_0 + (r_1^{\mathrm{c}} - r_1^{\mathrm{f}})\left\{\frac{NP_0 + L_0 + K^{-1} - \sqrt{(NP_0 + L_0 + K^{-1})^2 - 4L_0P_0}}{2}\right\}\right] \quad (1)$$

where N is the number of independent and equivalent interaction sites, K is the association constant, P_0 is the protein concentration, L_0 is the concentration of the paramagnetic complex, and r_1^{c} and r_1^{f} are the relaxivities expressed in $\mathrm{s}^{-1}\,\mathrm{mM}^{-1}$ of the complex HSA–contrast agent and of the free contrast agent, respectively.

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