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MULTINUCLEAR MR CHARACTERIZATION OF A NEW HEPATOBILIARY CONTRAST AGENT

Preliminary results

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Abstract

Purpose: The aim of this work was to characterize the hepatobiliary contrast agent Gd-EOB-DTPA in various media: water solution, protein solution, phosphorylated metabolite solution, and excised and perfused liver tissue.

Material and Methods: Different NMR techniques were used: analyses of H-1 NMRD profiles, H-2 NMR relaxation rates, O-17 relaxation rates and chemical shifts, and P-31 relaxation rates and peak area.

Results: The higher proton relaxivity of Gd-EOB-DTPA in water as compared to that of Gd-DTPA is due to a smaller distance r and to a longer τ_R. The kinetic stability of the former compound in ATP solution is higher and it forms noncovalent bonds with human serum albumin. Internalization of the contrast agent by the hepatocytes does not impair the ATP metabolism of the cells but induces relaxation effects on the intracellular metabolites of the liver.

Conclusion: Multinuclear MR studies allow the extensive characterization of MR contrast agents in *in vitro* and *ex vivo* model systems.

Key words: Hepatobiliary system, MR imaging; contrast media, Gd-EOB-DTPA; relaxivity.

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Gd-EOB-DTPA (gadolinium ethoxybenzyldiethylenetriaminepentaacetic acid (Eovist) is an amphiphilic MR contrast agent with hepatobiliary specificity. It is excreted by the kidneys and the bile (8, 10, 15) and its safety in humans has been demonstrated (5, 9). As compared to its more hydrophilic analog Gd-DTPA, Gd-EOB-DTPA has a higher proton relaxivity in water but the reason for this difference has not been elucidated (10, 15) while in plasma, the binding to proteins was related to the relaxivity enhancement (10, 15).

This work reports: i) some physicochemical characterization of the compound in water, in serum and proteincontaining solution; and ii) its stability in solutions containing ATP. Different heteronuclear NMR techniques were used: H-1 nuclear magnetic relaxation dispersion (NMRD), H-2 relaxation rates, O-17 chemical shifts and transverse relaxation rates, and P-31 relaxation rates. The effects of the hepatocellular uptake of Gd-EOB-DTPA on the ATP metabolism and on the relaxation of the phosphorylated metabolites were also analyzed on the excised and perfused rat liver model.

Material and Methods

Gd-EOB-DTPA and EOB-DTPA were provided by Schering AG. H-1 NMRD profiles were recorded on a Field Cycling System relaxometer (FCS, Oradell, USA) working between 0.24 mT and 1.2 T. H-1 relaxation rates were also measured at 0.47 T on a minispec Bruker PC 20, at 4.7 T on a spectrometer Bruker MSL-200-15 and at 7.05 T on a Bruker AMX-300 system. H-2, O-17 and P-31 NMR measurements on solutions were performed on the Bruker MSL-200 spectrometer. P-

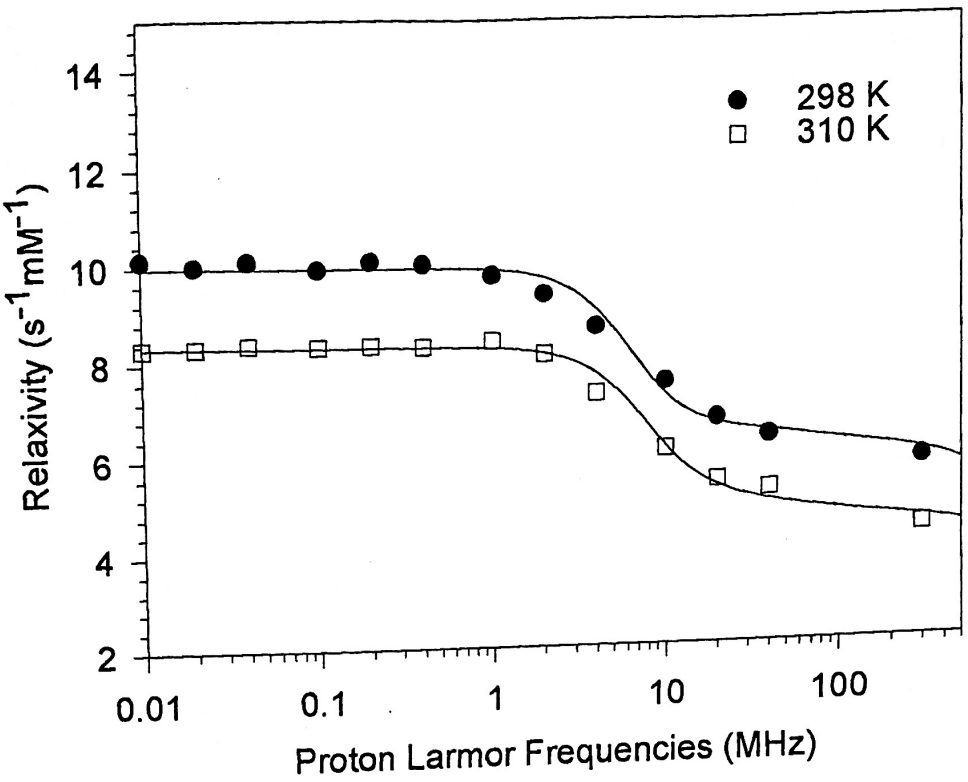

Figure 1. H-1 NMRD profiles of Gd-EOB-DTPA in water at different temperatures. The lines correspond to the theoretical fittings of the data points.

31 spectra of the liver were recorded on the Bruker AMX-300 spectrometer. Temperature was controlled by using air or nitrogen gas flow controlled by a BVT 1000 or BVT 2000 Bruker unit. Concentrations used for relaxation rates measurements did not exceed 50 mM. H-2 measurements were performed on the diamagnetic lanthanum complex of the ligand deuterated in alpha of the carboxylic groups (16). O-17 chemical shifts were measured on the dysprosium complex solution containing an excess of ligand. The excision and perfusion of the rat liver are described elsewhere (3). Male Wistar rats of 200–250 g b.w. were used. The protocol consisted of 10 min of control perfusion in a nonrecirculating mode, 20 min of perfusion with Gd-EOB-DTPA 1 mM dissolved in 200 ml of Krebs-Henseleit buffer free of EDTA (recirculating mode) and 40 min of washout (nonrecirculating mode). Two kinds of spectra were recorded: "relaxed" spectra with TR 3 s, pulse angle 60° and NA 50, or "saturated" spectra with TR 80 ms, pulse angle 60° and NA 1750.

Results and Discussion

Water solution: As compared to Gd -DTPA, the proton relaxivity of Gd-EOB-DTPA is higher over the whole frequency range (Fig. 1). The inner sphere model described by SOLOMON (11) and BLOEMBERGEN (2) and the outer sphere contribution described by FREED (4) are commonly used to analyze these NMRD profiles. Because of the large number of parameters used in these models, a nonambiguous analysis of the profiles requires the estimation of some of them by other techniques.

We used the method developed by ALPOIM et al. (1) to measure the number of coordinated water molecules (q). The chemical shifts induced on the O-17 resonance of water by dysprosium chloride, for which q is assumed to be equal to 8, are compared to those induced by Dy-EOB-DTPA complex. The measurements indicate that the q-value of Dy-EOB-DTPA is equal to 1.15 at 310 K.

The residence time of water molecules in the first coordination sphere (τ_M) was obtained from the analysis of the temperature dependence of the transverse relaxation rates of O-17 of water in Gd-EOB-DTPA solutions (7). The analysis of the experimental data obtained on a solution containing 50 mM of

Table 1

Values of τ_R, τ_{SO} and τ_V obtained from the fitting of the proton NMRD profiles at 298 and 310 K

	τ_R, ps	τ_{SO}, ps	τ_V, ps
T = 298 K	84	69	20.3
T = 310 K	61	63	17.1

Gd-EOB-DTPA gave values of τ_M equal to 83 ns and 201 ns at 310 and 298 K respectively.

In addition to these estimations of q and τ_M, we used H-2 longitudinal relaxation rates of the diamagnetic lanthanum complex labeled with 10 deuterium atoms to calculate the rotational correlation time of the complex. The relaxation rate of the deuterium nuclei is dominated by the intramolecular quadrupolar mechanism which is modulated by the rotation of the molecule only (6, 14). In the extreme narrowing conditions, the relaxation rate is directly related to the rotational correlation time (Eqn. 1).

$$\frac{1}{T_1(^2H)} = \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_R \qquad \text{(Eqn. 1)}$$

The quadrupolar coupling constant ($-e^2qQ/h-$) depends on the hybridization sate of the carbon atom linked to the deuterium and is available from the literature (6). H-2 R1 measurements of the diamagnetic lanthanum complex give values of τ_R equal to 93±12 ps and 66±9 ps at 298 and 310 K respectively.

Using the values of q and τ_M obtained above, we fitted the H-1 NMRD profiles. The relative diffusion constant D was assumed to be equal to the diffusion constant of water, d the distance of closest approach was fixed to 0.36 nm and the distance r was fixed at 0.281 nm, a value obtained by molecular modeling. Other parameters were optimized. The results of the fitting at 310 and 298 K are shown in Table 1. The values of τ_R are in very good agreement with those calculated from deuterium relaxation rate measurements. The higher relaxivity of Gd-EOB-DTPA as compared to Gd-DTPA is obviously related to a shorter distance r and to a longer τ_R whereas τ_M, q, τ_{SO} and τ_V are similar to those obtained for Gd-DTPA (12). These results are in good agreement with those reported for Gd-BOPTA, another aromatic derivative of Gd-DTPA (12).

Solutions containing phosphorylated metabolites: Through T1 measurements of the phosphorus nuclei of ATP in solutions containing Gd-DTPA and Gd-DTPA-BMA, we previously showed that ATP competes efficiently for Gd ions to form a complex between ATP and Gd (13). Similar experiments were performed with Gd-EOB-DTPA. The equilibrium value of the P-31 relaxation rates were similar for Gd-DTPA and Gd-EOB-DTPA but the steady state was reached after 30 min for Gd-DTPA and 6 h for Gd-EOB-DTPA. The kinetics of decomplexation is thus much slower for Gd-EOB-DTPA but the thermodynamic stability of both complexes is similar.

Serum and protein solutions: The H-1 NMRD profile of Gd-EOB-DTPA dissolved in serum shows at around 20 MHz a hump characteristic of an interaction with macromolecules. The interaction between the ligand and serum proteins was confirmed by a 17 Hz increase of the H-2 linewidth of the deuterated ligand (50 mM) in serum solutions as compared to water. Similarly, we observed an increase of deuterium linewidth (33 Hz) of the ligand dissolved in human serum albumin 4% when ligand concentration is decreased from 50 mM to 5 mM. Fitting of the data over the concentration range allows the estimation of the dissociation constant characterizing the interaction between HSA and EOB-DTPA ($K_d \cong 4$ mM) as well as the number of sites of interaction ($N \cong 1$).

The interaction between the Gd complex and HSA was investigated by proton relaxometry. The dissociation constant obtained by proton relaxometry ($K_d = 1.1$ mM) and the proton NMRD profiles of different concentrations of Gd-EOB-DTPA (0.07 mM to 1.06 mM) in HSA 4% at 310 K were used to calculate the theoretical proton NMRD profile of the complex HSA-(Gd-EOB-DTPA). This profile could be reasonably fitted assuming that q and r are unchanged but that the diffusion constant is shorter because of viscosity changes. The fitted value of τ_R (7 ns) is in good agreement with the size of albumin.

Excised and perfused rat livers: No marked change of ATP content was noticed during and after the perfusion of Gd-EOB-DTPA in relaxed spectra, showing that Gd-EOB-DTPA does not impair the ATP metabolism. Nevertheless, the linewidth of ATP peaks increased during perfusion of Gd-EOB-DTPA (maximum increase of 14 Hz) then decreased during the washout period to reach control level after 40 min of washout. Peak height increases were clearly seen on saturated spectra for phosphomonoester, phosphodiester and inorganic phosphate peaks confirming the influence of Gd-EOB-DTPA on T1 of slowly relaxing intracellular metabolites. All these results clearly show that Gd-EOB-DTPA enters the hepatocytes during Gd-EOB-DTPA perfusion and is removed from the liver cells during washout.

Conclusion

The higher relaxivity of Gd-EOB-DTPA in water as compared to Gd-DTPA can be explained by a shorter distance between the protons of the coordinated water molecule and the paramagnetic ion and by a longer rotational correlation time. The thermodynamic stability of both complexes is identical but the kinetic stability of Gd-EOB-DTPA is stronger.

The interaction of Gd-EOB-DTPA with serum proteins and more particularly with HSA was evidenced by proton NMRD and deuterium relaxation rates analysis and the dissociation constant characterizing the equilibrium was found to be in the millimolar range (1–4 mM). Finally, the P-31 NMR spectra of the isolated rat liver perfused with Gd-EOB-DTPA clearly confirms: i) the uptake of Gd-EOB-DTPA by hepatocytes through its relaxation effects on phosphorylated intracellular metabolites; ii) its washout from the liver cells; and iii) the absence of metabolic impairment consecutive to the Gd-EOB-DTPA uptake.

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