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Gd-EB-DTPA, a new dye-based MRI contrast agent

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Authors: S. Laurent[1], C. Burtea[1], L. Vander Elst[1], T. Yamamoto[2], H. Shimokawa[2], Y. Katayama[2], R.N. Muller[1]; [1]Mons/BE, [2]Fukuoka/BE

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1. Purpose

Recently, a new dye-based MRI contrast agent, Gd-EB-DTPA (EB = Evans Blue), has been synthesized [1] ([ESMRMB_EB-DTPA_Fig1.jpg] Figure 1). This compound, which is potentially useful for the detection of vascular endothelial injury, was found to selectively bind to endothelial lesions in excised porcine aorta or to the carotid artery of the living rat [2]. However, the precise mechanism responsible for this recognition is not yet elucidated. Based on the reported observations, we have tried to go further with the characterization of the contrast agent by evaluating its relaxometric properties, combined with protein binding ability, pharmacokinetic and biodistribution profile, which could explain, at least partially, the mechanism of blood retention and vascular targeting.

2. Methods and Materials

Physicochemical characterization NMRD profile was recorded on a field cycling relaxometer (Stelar, Italy).The relaxivity of Gd-EB-DTPA in HSA solution (4%) was determined by proton longitudinal relaxation rate (R_1) measurement at 0.47 T.Blood pharmacokinetics Plasma pharmacokinetics were assessed on male Wistar rats anesthetized with 50 mg Nembutal/kg b.w., i.p. The rats were tracheotomized, and the left carotid artery was catheterized for blood collection. The contrast agent was injected as a bolus through the femoral vein at a dose of 0.05 mmol/Kg b.w. Gd-DTPA, used as a control, was injected at a dose of 0.1 mmol/Kg b.w. Blood samples were collected (~0.3 mL, with saline replacement) before and at 1, 2.5, 5, 15, 30, 45, 60, 90 and 120 min after injection. The gadolinium content of the blood samples was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Jobin Yvon JY70+, Longjumeau, France) after mineralization by microwaves (Milestone MSL-1200, Sorisole, Italy) in acidic conditions (0.6 mL HNO_3, 0.3 mL H_2O_2). A two-compartment distribution model was used to calculate the pharmacokinetic parameters such as the elimination half-life ($T_{e1/2}$), the steady state volume of distribution (VD_{ss}), the total clearance (Cl_{tot}), and the initial blood concentration (C_0).Biodistribution The biodistribution has been determined in male Wistar rats 2 h after a single i.v. injection of 0.05 mmol Gd/kg bw. The organs (liver, kidneys, heart, spleen, lungs) were weighted, dried overnight at 60°C, and subsequently digested (up to 0.4 g /sample) in acidic conditions (3 ml HNO_3, 1 ml H_2O_2) by microwaves. The gadolinium content was determined by ICP as described above and expressed as percentage of the injected dose / g (% of ID/g).

3. Results

Relaxometric study The NMRD profile of Gd-EB-DTPA is shown in [ESMRMB_EB-DTPA_Fig2.jpg] Figure 2. The longitudinal relaxivity (r1) at high magnetic fields is particularly high for such compound of low molecular weight (1151 g/mol). This characteristic and the shape of the NMRD profile suggest the presence of supramolecular aggregates. To verify this possibility, the r1 and r2 (transverse relaxivity) were measured at two magnetic fields (60 and 300 MHz) and at 37°C as a function of Gd-EB-DTPA concentration ([ESMRMB_EB-DTPA_Fig3.jpg] Figure 3). The r1 values of Gd-EB-DTPA are remarkably high and concentration dependent, ranging between 4.9 s^{-1} mM^{-1} (0.33 mM) and 12.2 s^{-1} mM^{-1} (2.37 mM) at 60 MHz. This latter value is more than threefold increased as compared to traditional MRI contrast agents like Gd-DTPA (3.4 $s^{-1}mM^{-1}$ at 60 MHz). This behavior may be related to a reduced motion explained by supramolecular aggregates. The presence of micelles was confirmed by photocorrelation spectroscopy (PCS) measurements.Based on the measurements shown in [ESMRMB_EB-DTPA_Fig3.jpg] Figure 3, the ratio r2/r1 was calculated and the values are shown in [ESMRMB_EB-DTPA_Table1.jpg] Table 1. At clinical magnetic field (60 MHz), they are generally close to 1, which indicate a good potential for both T1- or T2-weighting in MRI. Although the relaxivity of

Gd-EB-DTPA is concentration-dependent, its value is enhanced in HSA solution (R_1^p (HSA) / R_1^p(w) = 1.9 as compared to 1.2 for Gd-DTPA) ([ESMRMB_EB-DTPA_Table2.jpg] Table 2), indicating a noncovalent interaction. This interaction is however weaker than that of MS-325, which has a relaxivity 4.6 times higher in the presence of HSA than in aqueous solution.Blood pharmacokinetics The blood pharmacokinetic profile of Gd-EB-DTPA was evaluated in Wistar rats ([ESMRMB_EB-DTPA_Fig4.jpg] Figure 4) and compared with that of Gd-DTPA, the parent compound. It is remarkable that the blood concentration of Gd-EB-DTPA 2 h after administration is significantly higher (40% of C_0) than that of Gd-DTPA, which is already cleared off at this time.The pharmacokinetic parameters were calculated by fitting the blood concentration curves as a function of time with a bi-exponential equation ([ESMRMB_EB-DTPA_Table3.jpg] Table 3). The results ($T_{e1/2}$ = 160 min, Cl_{tot} = 0.82 mL/kg/min) indicate that the blood clearance of Gd-EB-DTPA is significantly slower than that of Magnevist® ($T_{e1/2}$ = 15 min, Cl_{tot} = 8.66 mL/kg/min) and is probably related to albumin binding. However, albumin binding is not the only one responsible for the delayed blood clearance, since MS-325 has a higher binding capacity, while its $T_{e1/2}$ is of only 22.5 min in the same species and at the same dose [3]. The VD_{ss} (0.185 L/kg) is not significantly different from that of Magnevist® (0.165 L/kg). The delayed blood clearance could point out Gd-EB-DTPA as a new blood pool contrast agent beside its ability to specifically target the injured vascular endothelium.Biodistribution The biodistribution in different organs (liver, kidneys, heart, spleen, lungs) of Gd-EB-DTPA was evaluated in Wistar rats 2 h after the contrast agent administration ([ESMRMB_EB-DTPA_Fig5.jpg] Figure 5). The results are compared to the biodistribution of Gd-DTPA. With the exception of the heart, Gd-EB-DTPA is accumulated ($p<0.01$) in organs characterized by a network of fenestrated or sinusoid capillaries [4, 5]. This could mean that the compound can diffuse in the interstitial space. This phenomenon is limited however by the retention into the blood pool as a result of an interaction with HSA.

4. Conclusion

The remarkably high molecular relaxivity of Gd-EB-DTPA in water solution subsequent to the formation of supramolecular aggregates and its interaction with HSA highlight the compound as a possible contrast agent dedicated to MR angiography. This quality is supported by the delayed blood clearance, which could be an additional advantage for molecular imaging of the damaged vascular endothelium.

5. References

1. T. Yamamoto et al, Anal. Sci, 20, 5 (2004).
2. T. Yamamoto et al, Bioorg. Med. Chem. Letters, 14, 2787-2790 (2004).
3. Parmelee DJ et al, Invest Radiol, 32, 741-747 (1997).
4. Okuhata Y, Advanced Drug Delivery Reviews, 37, 121-137 (1999).
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6. Personal Information

Acknowledgements

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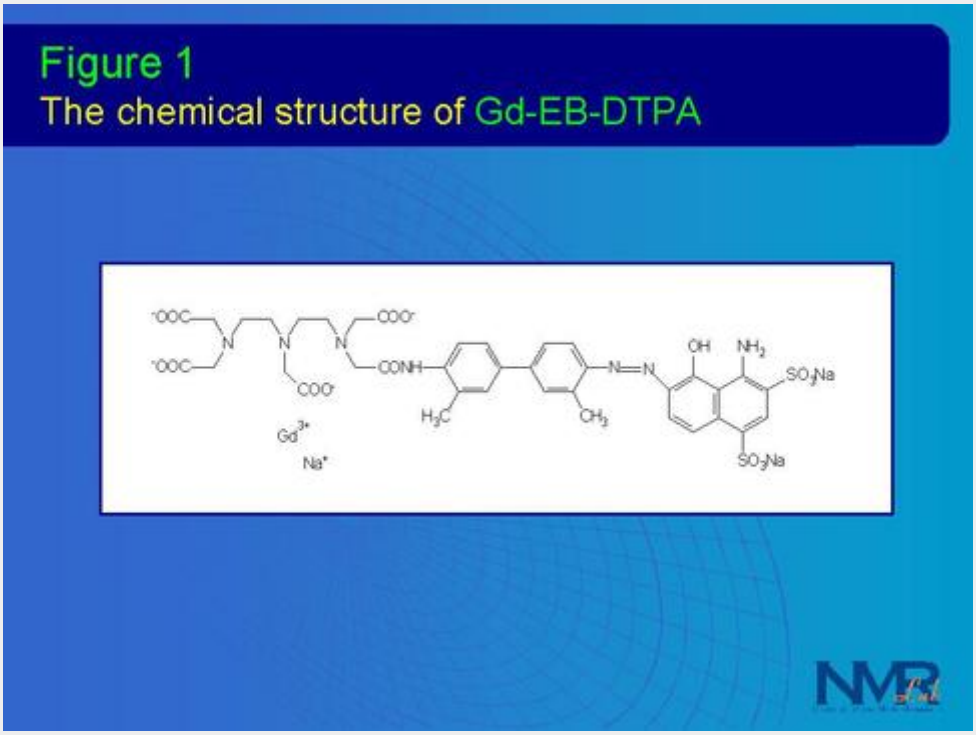
7. Mediafiles

ESMRMB_EB-DTPA_Fig1.jpg



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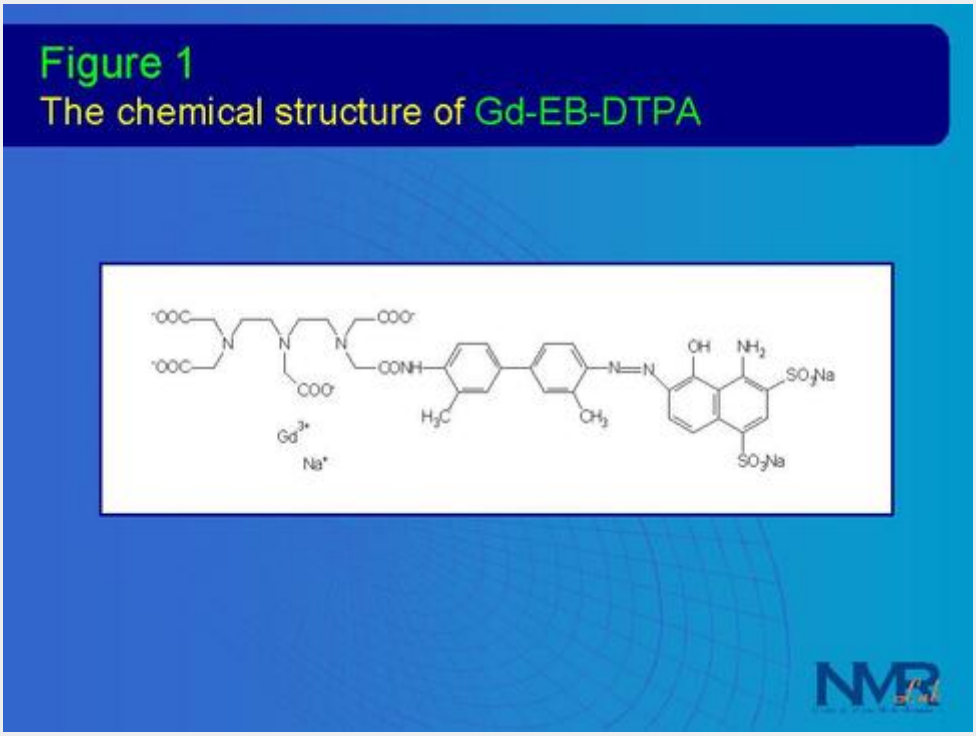

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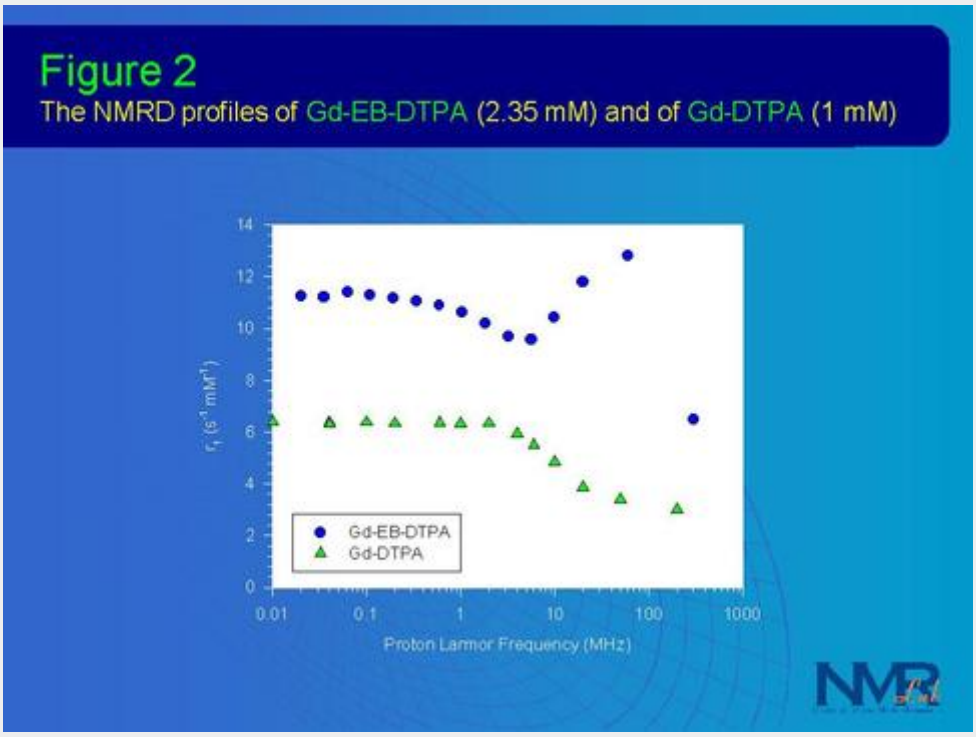

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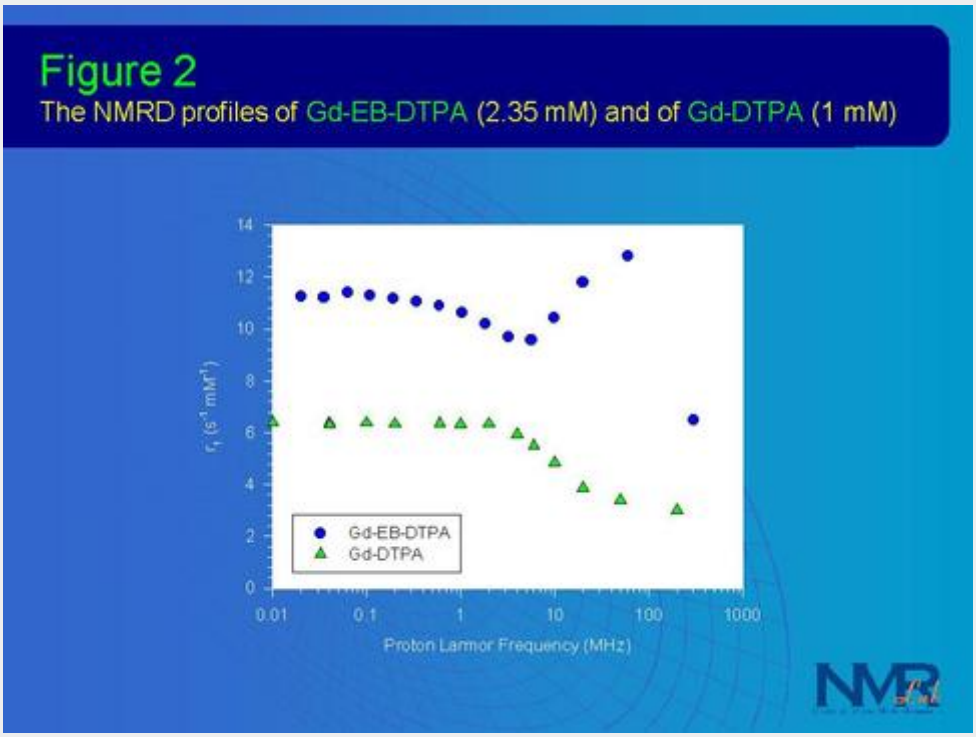

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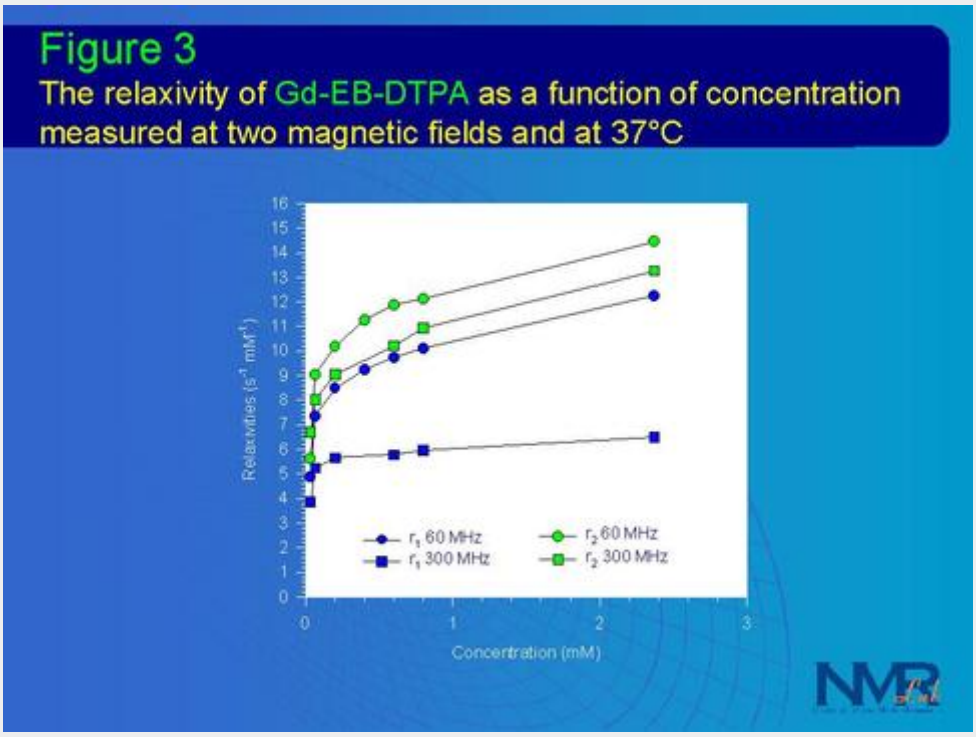

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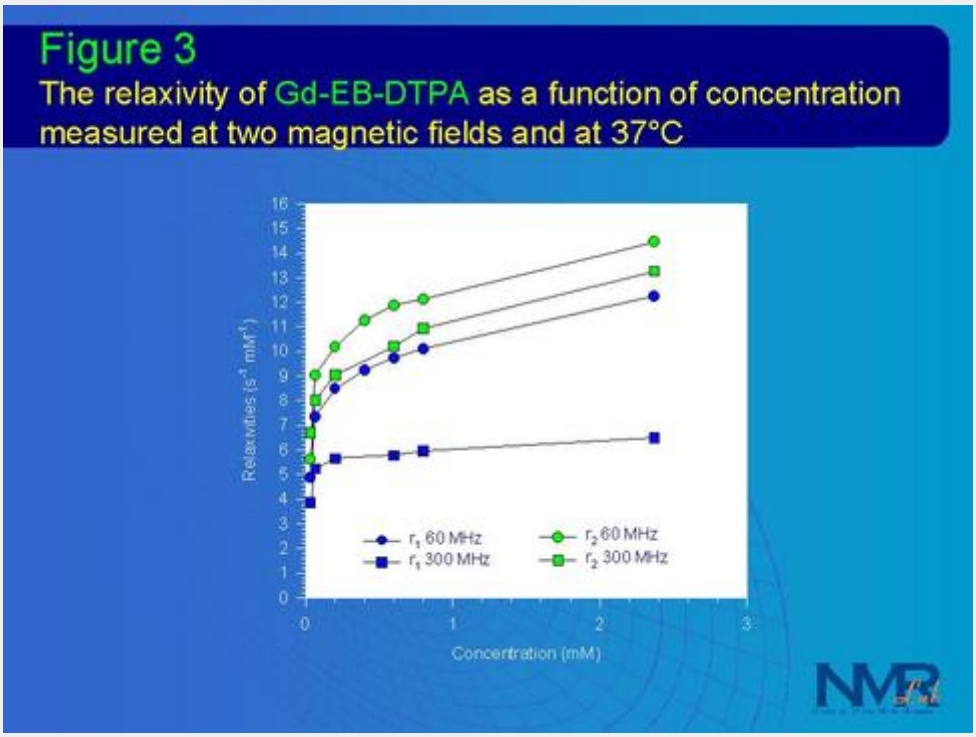

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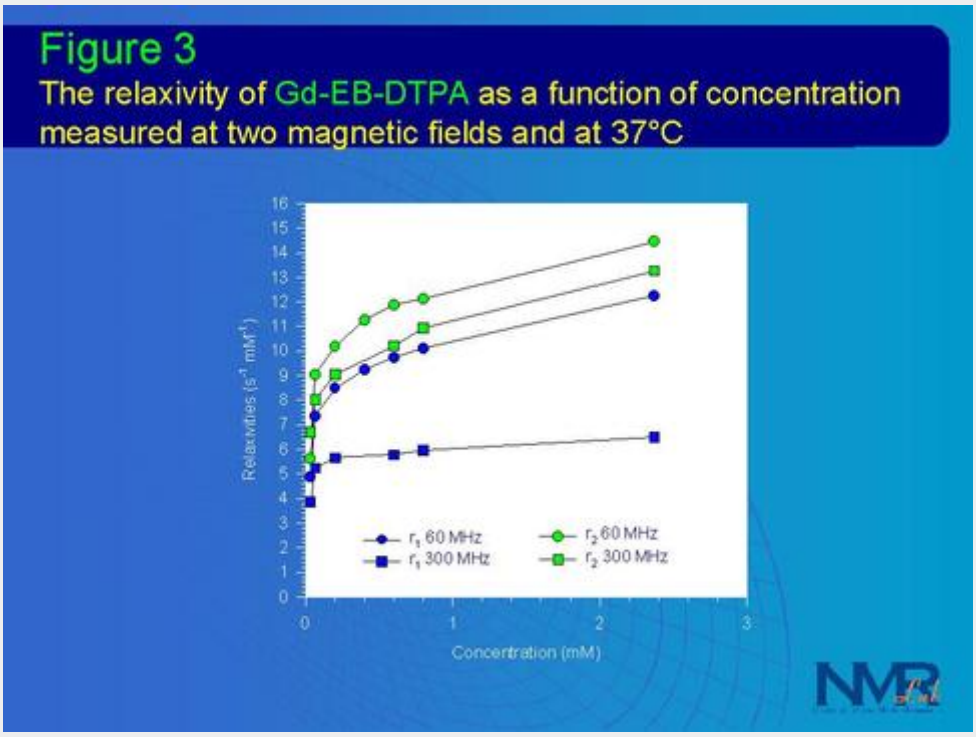

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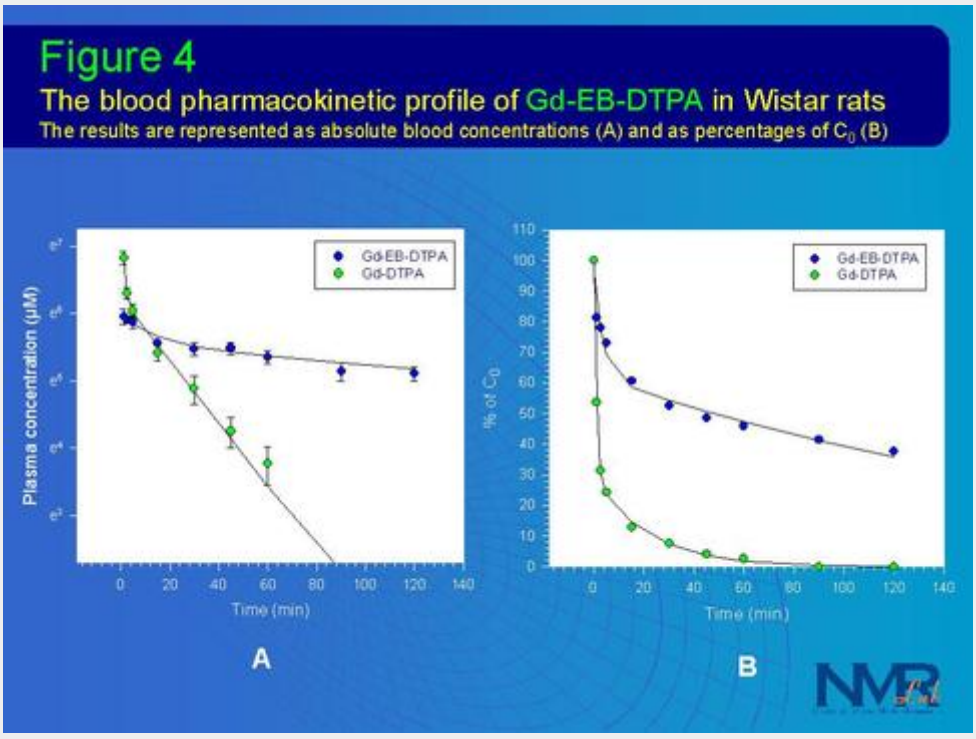

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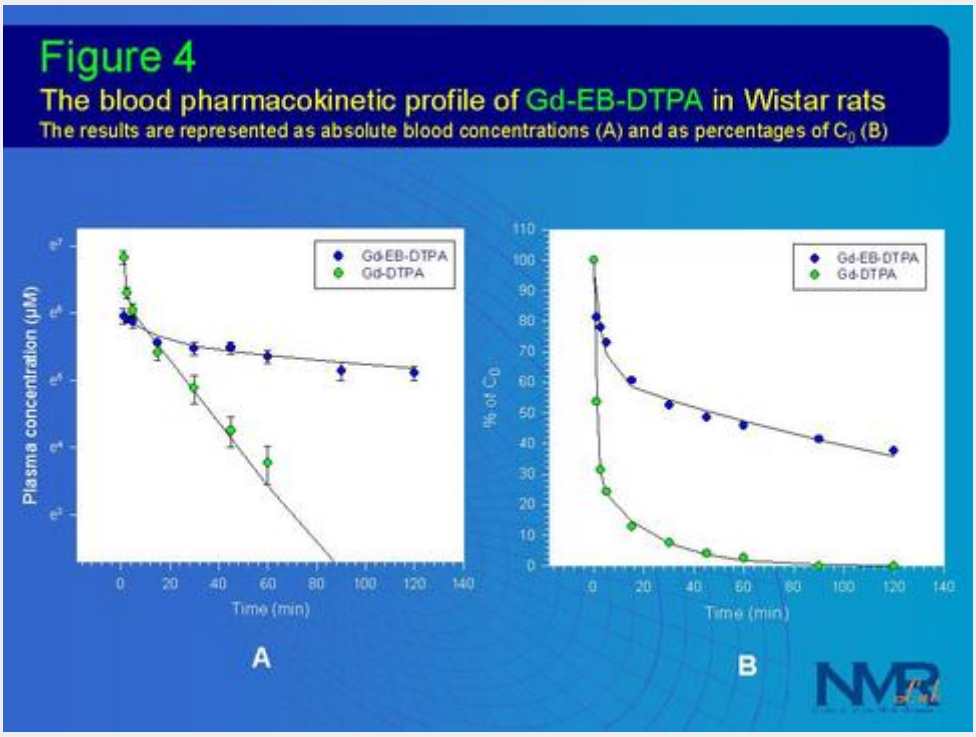
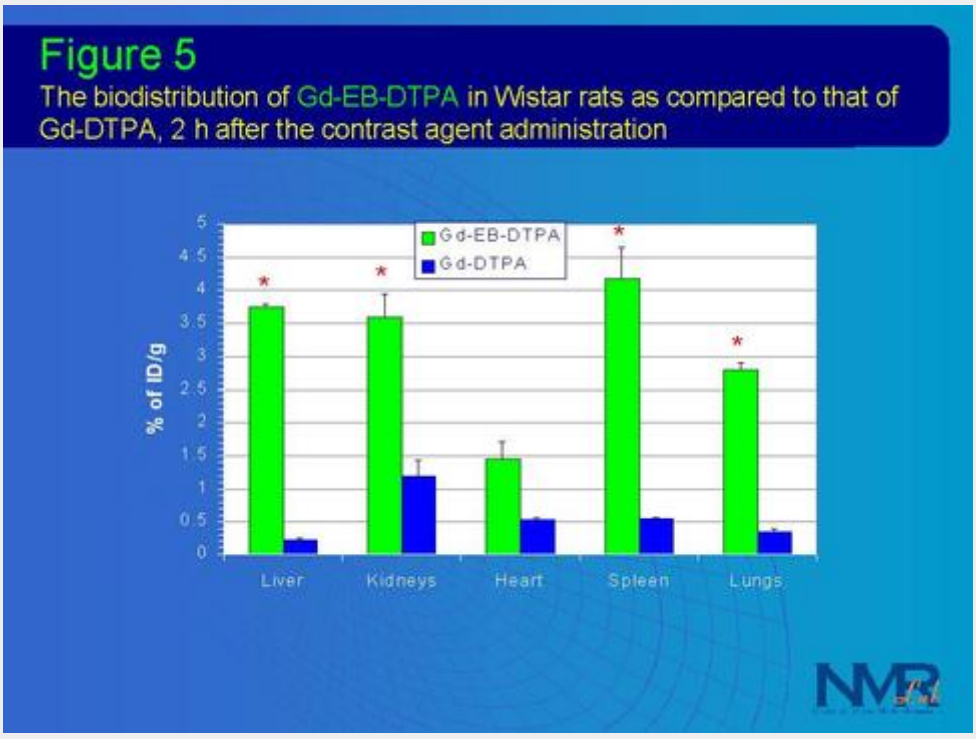


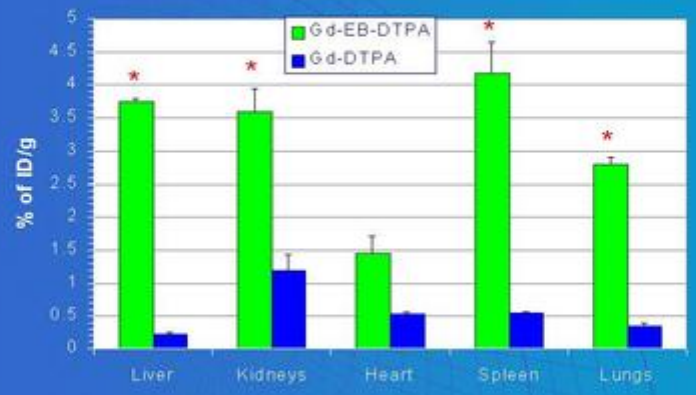
ESMRMB_EB-DTPA_Fig5.jpg



ESMRMB_EB-DTPA_Fig5.jpg

Figure 5

The biodistribution of Gd-EB-DTPA in Wistar rats as compared to that of Gd-DTPA, 2 h after the contrast agent administration



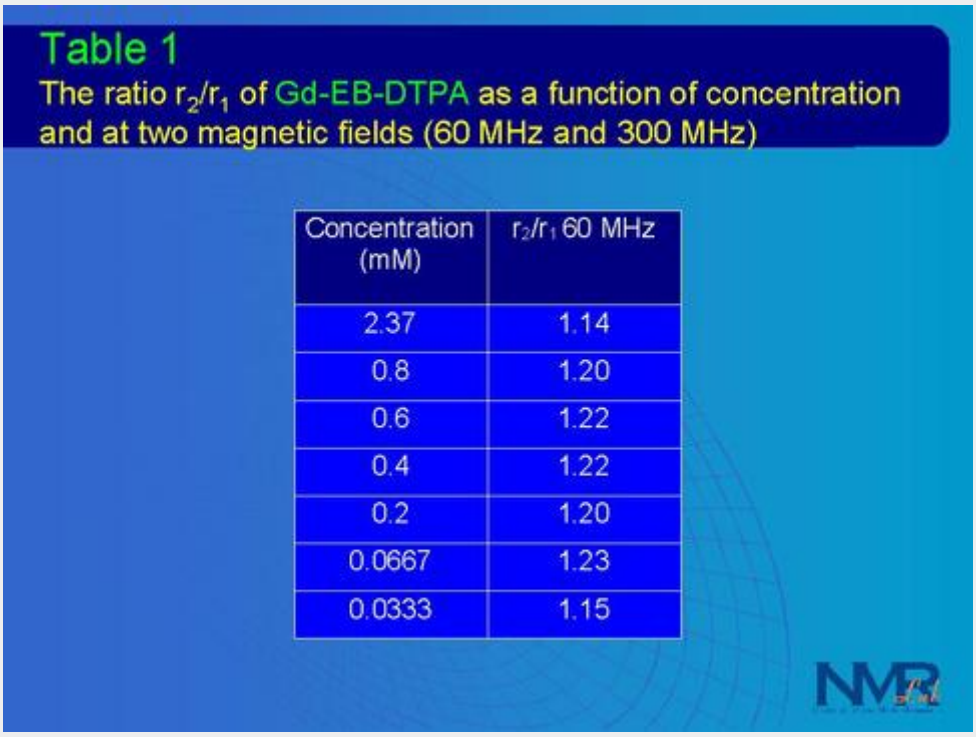
ESMRMB_EB-DTPA_Table1.jpg

Table 1

The ratio r_2/r_1 of Gd-EB-DTPA as a function of concentration and at two magnetic fields (60 MHz and 300 MHz)

Concentration (mM)	r_2/r_1 60 MHz
2.37	1.14
0.8	1.20
0.6	1.22
0.4	1.22
0.2	1.20
0.0667	1.23
0.0333	1.15

ESMRMB_EB-DTPA_Table1.jpg



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ESMRMB_EB-DTPA_Table2.jpg

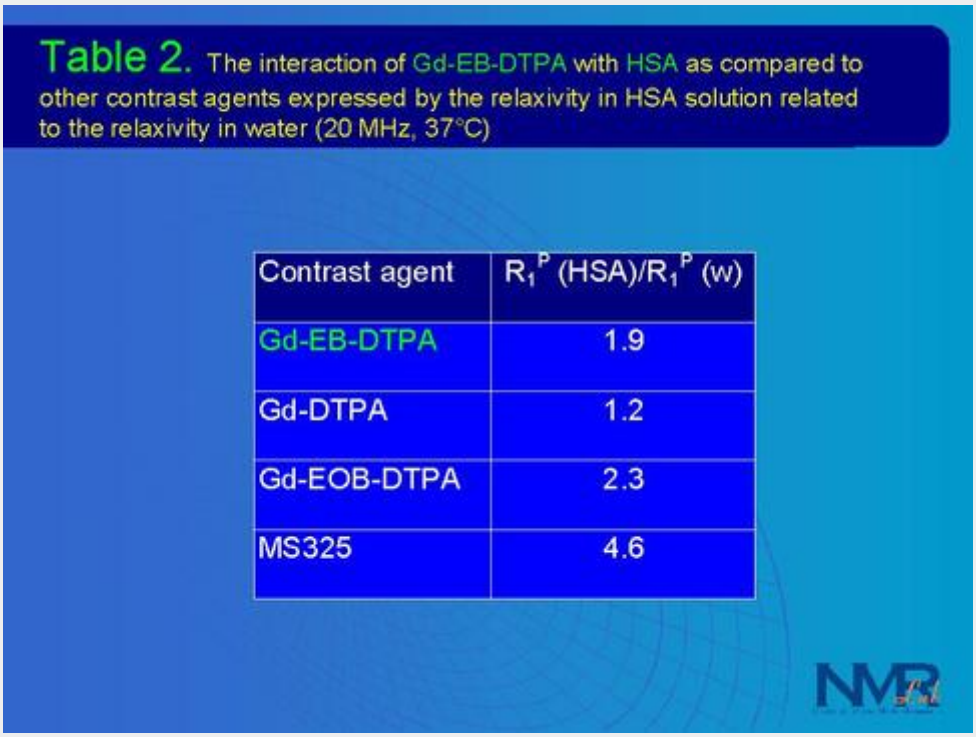

Table 2. The interaction of Gd-EB-DTPA with HSA as compared to other contrast agents expressed by the relaxivity in HSA solution related to the relaxivity in water (20 MHz, 37°C)

Contrast agent	R_1^P (HSA)/R_1^P (w)
Gd-EB-DTPA	1.9
Gd-DTPA	1.2
Gd-EOB-DTPA	2.3
MS325	4.6

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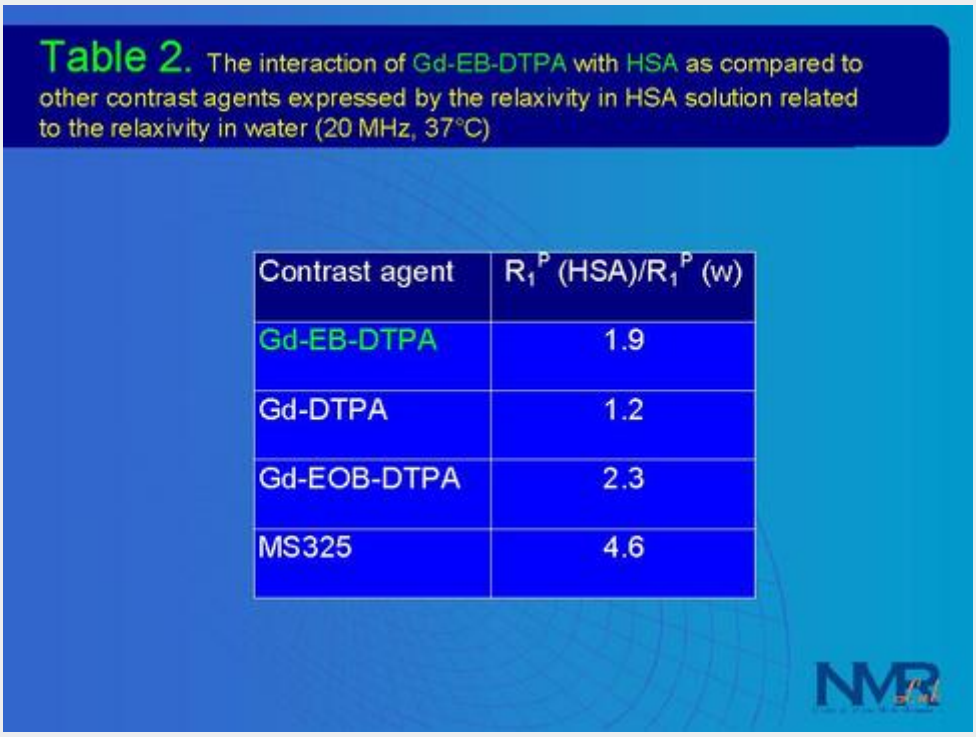

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Gd-EB-DTPA	1.9
Gd-DTPA	1.2
Gd-EOB-DTPA	2.3
MS325	4.6

ESMRMB_EB-DTPA_Table3.jpg

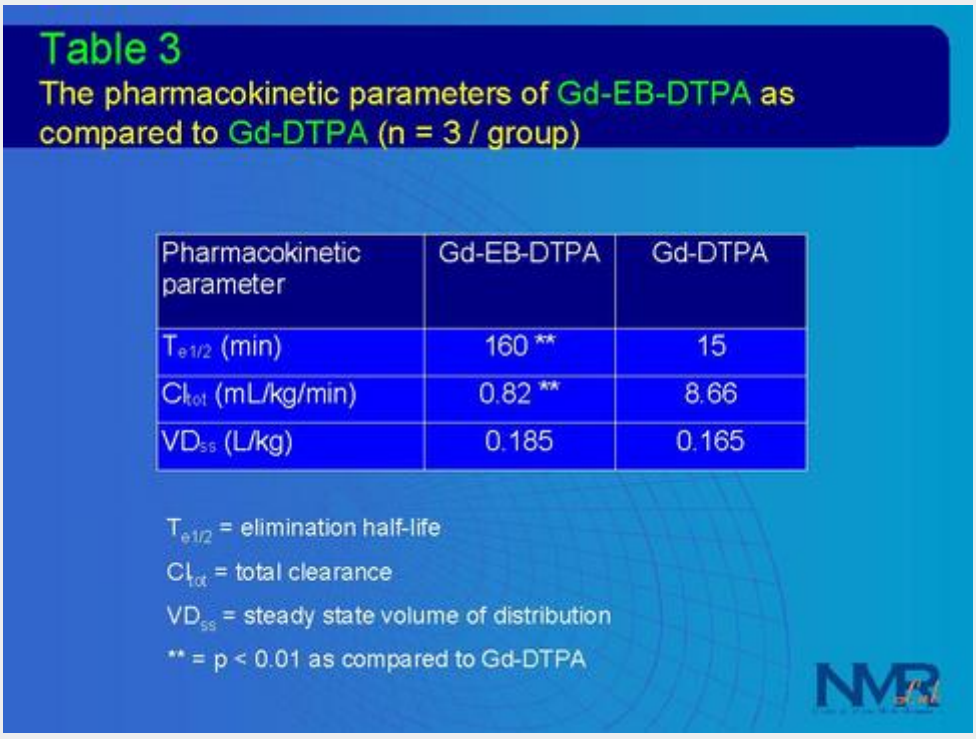
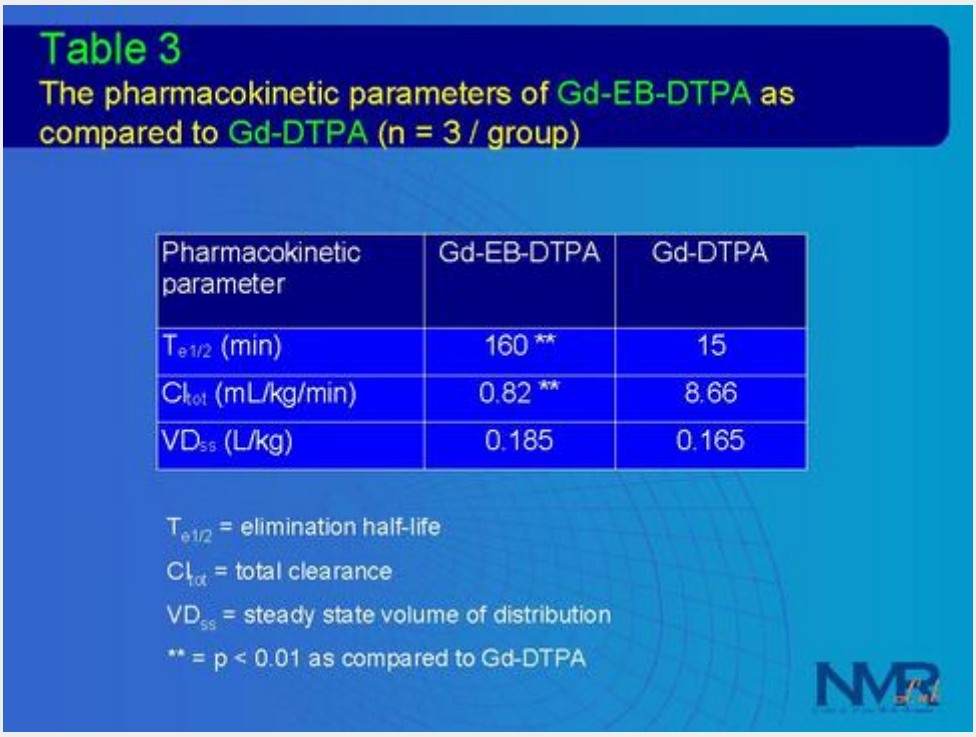


Table 3
The pharmacokinetic parameters of Gd-EB-DTPA as compared to Gd-DTPA (n = 3 / group)

Pharmacokinetic parameter	Gd-EB-DTPA	Gd-DTPA
$T_{e1/2}$ (min)	160 **	15
Cl_{tot} (mL/kg/min)	0.82 **	8.66
VD_{ss} (L/kg)	0.185	0.165

$T_{e1/2}$ = elimination half-life
Cl_{tot} = total clearance
VD_{ss} = steady state volume of distribution
** = $p < 0.01$ as compared to Gd-DTPA

ESMRMB_EB-DTPA_Table3.jpg



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