Nuclear spin relaxation of liposomal paramagnetic contrast agents

G. J. Strijkers[1], R. B. van Heeswijk[1], W. J. Mulder[1], P. C. Magusin[2], P. M. Frederik[3], P. Bomans[3], K. Nicolay[1]

[1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, Eindhoven, Netherlands, [3]Department of Pathology, EM Unit, Maastricht University, Maastricht, Netherlands

Introduction

Chelates of Gd^{3+}, such as Gd-DPTA and GD-DOTA, are commonly used contrast agents for MRI. However, the field of molecular MR imaging, which aims to detect sparse concentrations of molecular epitopes, has given rise to a strong demand for more effective T_1 lowering contrast agents, *i.e.* with a high relaxivity. Potential candidates are highly potent contrast agents based on paramagnetic liposomes, because they can carry a large payload of Gd-chelate in the lipid bilayer. This leads to an enormous increase of the relaxivity per particle, which lowers the detection limit of these contrast agents dramatically as compared to for example Gd-DTPA [1].

The aim of this work was to characterize the relaxation properties of pegylated paramagnetic liposomal contrast agents, as a function of composition, temperature and magnetic field strength.

Materials & Methods

Liposome preparation: The liposomes were prepared in 4 formulations, differing in the fatty acid chain, saturated versus unsaturated, and the presence of cholesterol. The compositions were as follows: (*formulation A*) Gd-BSA (=Gd-DTPA-bis(stearylamide)), DSPC (saturated), PEG-DSPE, cholesterol, (*formulation B*) Gd-BSA, DSPC, PEG-DSPE, (*formulation C*) Gd-BOA (=Gd-DTPA-bis(oleyamide)), DOPC (unsaturated), PEG-DSPE, cholesterol, (*formulation D*) Gd-BOA, DOPC, PEG-DSPE. The liposomes were prepared by lipid film hydration and sized by extrusion through 400 and 100nm filters.

TEM: A suspension of the liposomes was contained in a thin film and vitrified by rapid cooling in a liquid coolant. Transmission electron microscopy (TEM) was subsequently performed at -170°C using an electron microscope (Philips CM12) operating at 120kV.

NMR: The temperature dependence of the relaxivity was measured with a Bruker MSL200 NMR spectrometer operating at 200MHz. T_1 was determined using an inversion recovery sequence with inversion times between 10ms and 3s. T_2 was measured using a spin-echo sequence with variable echo time ranging between 100µs and 300ms. Relaxivities (r_1 and r_2) were determined from linear fits of $1/T_1$ and $1/T_2$ as function of Gd-ion concentration for total lipid concentrations of 0.3, 0.9, and 1.2mM. The temperature was varied between 298 and 358K.

NMRD: Nuclear magnetic relaxation dispersion was performed on a Stelar Spinmaster FFC-2000 (Mede, Italy). Relaxivity r_1 was determined between 0.01 and 35MHz at temperatures of 293, 310, and 333K. Interpretation of the NMRD curves was done using the theory of nuclear spin relaxation in paramagnetic systems by Bertini et al. [2] and Kruk et al. [3].

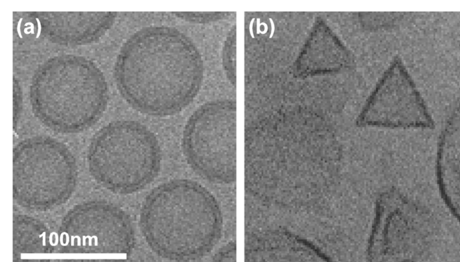

Figure 1: TEM images of Gd-BSA liposomes, (a) formulation A, with cholesterol and (b) formulation B, without cholesterol.

Results & Discussion

In figure 1 cryo-TEM images of formulation A and B are shown. Cholesterol addition leads to spherical bilayer particles, while triangular shaped vesicles are formed without cholesterol. Formulation C results in similar monodisperse spheres, while formulation D results in cigar-shaped vesicles (not shown).

The temperature dependences of r_1 and r_2 at 200MHz are shown in figure 2. The relaxivities for all formulations are higher than for traditional Gd-DTPA. Note that the relaxivities are reported in terms of Gd-ion concentrations and therefore the relaxivity per particle is much higher as a 100nm liposome may contain up to 50'000 Gd ions. Liposomes with cholesterol have a higher r_1 than those without cholesterol, probably as a consequence of an increase in the motional correlation times due to sterical hindering of the Gd-lipids by the cholesterol. The DPSC-based liposomes (A,B) display a maximum in r_1 around 335K, which coincides with a gel to liquid-crystalline phase transition, confirmed with differential scanning calorimetry. Similar temperature dependence is found for r_2. For the DOPC-based liposomes (C,D) the above phase transition lies below 273K and r_1 shows remarkably little temperature dependence at this field strength, although a large increase in r_2 with temperature is observed. At physiological temperature (310K) and 200MHz the r_2/r_1 ratios are 6.2, 9.3, 5.4, and 6.3 for formulations A, B, C, and D, respectively, which makes all formulations well suited as T_1 lowering contrast agents.

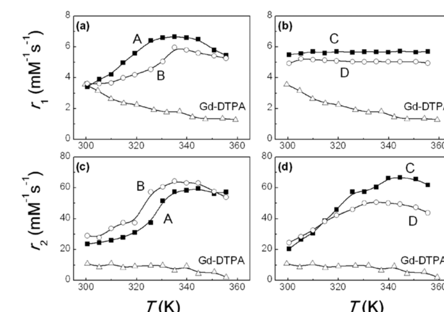

Figure 2: Temperature dependence of (a,b) r_1 and (c,d) r_2 for the different formulations (A, B, C, D) and Gd-DTPA at 200MHz.

Relaxivity dispersion curves for the formulations A and C are shown in figure 3. For both the relaxivity increases with temperature, indicating that the relaxation is limited by the water exchange [4]. We infer that the Gd at the inner leaflet of the membrane particles becomes more accessible to water at higher temperatures, thus contributing more to the relaxivity. This is confirmed by the fact that the DOPC-based liposomes (C) have a higher relaxivity, since they are in the liquid-crystalline phase for all temperatures and thus have a less rigid membrane, more permeable to water. A peak in relaxivity is observed for all curves at around 30MHz, controlled by the onset of electronic relaxation processes.

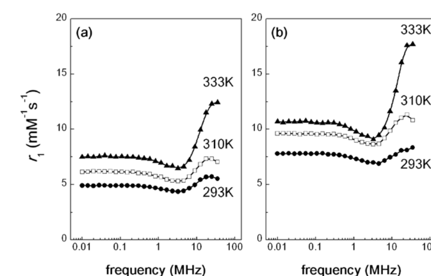

Figure 3: Frequency dependence of r_1 for (a) liposomal formulation A and (b) liposomal formulation C at T=293, 310, and 333K.

Conclusions

In summary, we have characterized liposomes with saturated and unsaturated phospholipids, and with and without cholesterol. Addition of cholesterol is necessary to obtain monodisperse unilamellar liposomes. The relaxivity of liposomes with unsaturated phospholipids is higher as compared to those with saturated lipids, because of higher accessibility of water. Cholesterol leads to a further, although smaller, increase of the relaxivity probably caused by an increase in the motional correlation times. The highest relaxivity of 11.3mM^{-1}s^{-1} at physiological temperature (310K) was obtained for DOPC-based liposomes with cholesterol at 25 MHz. These results show that paramagnetic liposomes are highly potent contrast agents, making them excellent candidates for many applications in molecular MR imaging.

References

[1] Mulder et al., Bioconjugate Chem. 15, 799 (2004).
[2] Bertini et al., J. Chem. Phys., 111, 5795-5807 (1999).
[3] Kruk et al., Phys. Chem. Chem. Phys. 3, 4907-4917 (2001).
[4] Tilcock et al., Magn. Reson. Med. 27, 44 (1992).